# SHIELD FOR DETECTION OF COLORECTAL CANCER

## SPONSOR EXECUTIVE SUMMARY

## MOLECULAR AND CLINICAL GENETICS PANEL

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***ADVISORY COMMITTEE BRIEFING MATERIALS:***
***AVAILABLE FOR PUBLIC RELEASE***

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## List of Abbreviations

| Abbreviation | Definition |
|---|---|
| AA | Advanced adenoma |
| AE | Adverse event |
| AGA | American Gastroenterological Association |
| AN | Advanced neoplasia |
| AUC | Area under the curve |
| AV | Analytical validation |
| BCK | Blood Collection Kit |
| BCT | Blood collection tube |
| CAN-SCREEN | Colorectal cANcer SCReening Economics and adherENce |
| cfDNA | Cell-free DNA |
| CI | Confidence interval |
| CISNET | Cancer Intervention and Surveillance Modeling Network |
| CLIA | Clinical Laboratory Improvement Amendments |
| CRC | Colorectal cancer |
| CRO | Contract research organization |
| CV | Clinical validation |
| ECLIPSE | Evaluation of the ctDNA LUNAR Test in an Average Patient Screening Episode |
| FDA | Food and Drug Administration |
| FIT | Fecal immunochemical test |
| FOBT | Fecal occult blood test |
| HGD | High-grade dysplasia |
| LDT | Laboratory developed test |
| LYG | Life-years gained |
| MR Score | Methylated Regression Score |
| mSept9 | methylated Septin 9 |
| mtsDNA | multi-target stool DNA |
| NCCN | National Comprehensive Cancer Network |
| NPV | Negative predictive value |
| PMA | Premarket Approval |
| PPV | Positive predictive value |
| ROC | Receiver Operating Characteristic |
| SAE | Serious adverse event |
| SEER | Surveillance, Epidemiology, and End Results |
| UADE | Unanticipated adverse device effect |
| US | United States |
| USPSTF | United States Preventive Services Taskforce |

# 1 SYNOPSIS

## 1.1 Introduction

Guardant Health is seeking approval of Shield™, a blood-based colorectal cancer (CRC) screening test for average-risk adults. Shield is an in vitro diagnostic device intended for the qualitative detection of CRC through sequencing the cell-free DNA (cfDNA) isolated from whole blood.

Despite the widespread availability of many CRC screening options, there are persistent barriers to screening completion, leading to only 58% of eligible individuals aged 45–75 being up to date with CRC screening (Siegel et al 2023). This rate is well below the target of 80% set by leading health organizations and leaves approximately 50 million screen-eligible Americans not up to date with CRC screening (Siegel et al 2023; Wender et al 2020). Screening reduces mortality from CRC. Importantly, 76% of CRC related deaths occur in individuals who are not up to date with CRC screening (Doubeni et al 2019). The persistently low rate of CRC screening in the population contributes to high rates of CRC-related deaths and CRC remains the second leading cause of cancer-related deaths in the US (Siegel et al 2024).

Shield has a proven level of performance for CRC detection (83.1%), within range of stool-based screening options and superior to the FDA approved blood-based screening test. In addition, initial real-world data from the Shield Laboratory Developed Test (LDT) demonstrates that Shield, as a convenient blood test that can be completed during any healthcare encounter, is more likely to be completed (defined as “adherence”) than existing screening modalities (Coronado et al 2024; Raymond et al 2023). Shield has limited performance for detection of advanced adenomas (AA) which limits impact on prevention of disease; however, when considering the multi-year transition from AA to CRC, which allows multiple test instances, Shield’s high early-stage CRC sensitivity and expected increased adherence to the first and subsequent Shield tests, the risk of accrued harm would be mitigated. In totality, Shield’s performance supports its utility as a primary CRC screening option alongside other screening modalities and when combined with high adherence to blood testing, can add to the impact of current CRC screening modalities on CRC mortality.

## 1.2 Background and Unmet Need

CRC is the fourth most diagnosed cancer and second leading cause of cancer-related death in the US (Siegel et al 2024). Current guidelines recommend CRC screening for average-risk individuals who are 45–75 years old, as detection of asymptomatic CRC results in substantial improvement in CRC mortality (Levin et al 2008; Patel et al 2022; Rex et al 2017; USPSTF et al 2021; Wolf et al 2018). An estimated 76% of CRC-related deaths occur in individuals who are not up to date with screening (Doubeni et al 2019).

CRC screening reduces CRC-related mortality through detection of early-stage asymptomatic CRC and / or identification and removal of pre-cancerous lesions (e.g.,

adenomas) which prevents the development of CRC. There are several CRC screening options available, including direct visualization tests (e.g., colonoscopy and sigmoidoscopy) and non-invasive stool-based tests (e.g., hsgFOBT, FIT, mtsDNA) with varying performance characteristics (Table 3). Screening guidelines recommend any approved screening test, stating that choice of screening test should depend on individual preference and test availability (USPSTF et al 2021).

However, suboptimal adherence with current screening options points to a pressing need for additional non-invasive CRC screening options that people will complete. The challenge with existing CRC screening options is that many individuals consider them burdensome. This leads to delayed or deferred screening, which contributes to high non-completion rates, leaving 50 million American adults unscreened for CRC.

Incorporating a blood-based test alongside currently existing CRC screening options provides additional choices. The incorporation of multiple choices up front in preventive care discussions has been shown to increase the likelihood that an individual completes the intervention (Inadomi et al 2012). A blood-based CRC screening test performed as part of a routine health care encounter would provide an additional screening option that is relatively simple to complete, thus improving adherence (Adler et al 2014; Liang et al 2023).

### 1.3 cfDNA as a Biomarker for Colorectal Cancer

One of the most promising approaches to detect the presence of tumor from blood is via the detection of tumor-derived DNA. During cellular death (e.g., apoptosis, necrosis), DNA is released from the cells into the circulation, forming cell free DNA (cfDNA). This cfDNA can then be found in the plasma compartment of whole blood. cfDNA fragments originating from the tumor carry the underlying genomic sequence mutations and epigenomic modifications of the tumor cells. Since colorectal tumors harbor a large number of somatic mutations and epigenomic modifications which typically arise early in colorectal neoplasia (Cancer Genome Atlas Network 2012; Hanley et al 2017; Lao and Grady 2011; Simmer et al 2012), tumor-derived cfDNA provides the opportunity to identify tumor presence by analyzing cfDNA accessible from a simple blood draw (Stroun et al 1989). Shield analyzes cfDNA for somatic mutations, cfDNA fragmentation patterns, and aberrant methylation across > 2,000 genomic regions to detect the presence of CRC.

### 1.4 Overview of Shield Test

The Shield test is a qualitative in vitro diagnostic test intended to detect colorectal cancer derived alterations in cell-free DNA from blood collected in the Guardant Shield Blood Collection Kit.

Shield is indicated for colorectal screening in individuals at average risk of the disease, age 45 years or older. Patients with an “Abnormal Signal Detected” may have colorectal cancer or advanced adenomas and should be referred for colonoscopy evaluation.

Shield is not a replacement for diagnostic colonoscopy or for surveillance colonoscopy in high-risk individuals. The test is performed at Guardant Health, Inc.

Guardant Health's Shield is a blood-based CRC screening test that detects CRC through sequencing of cfDNA, which carries the genomic and epigenomic alterations found in the tumor into the circulation.

Shield is a high throughput automated test performed in the Clinical Laboratory Improvement Amendments (CLIA) certified Guardant Health Clinical Laboratory. Shield includes a Blood Collection Kit (BCK) that is distributed for blood collection and then shipped to Guardant Health, reagents for assay processing, laboratory instrumentation with customized methods to automate the workflow, and software for sample processing, data analysis, and report generation. The Shield test returns a simple "Abnormal Signal Detected" or "Normal Signal Detected" result.

## 1.5 ECLIPSE Study

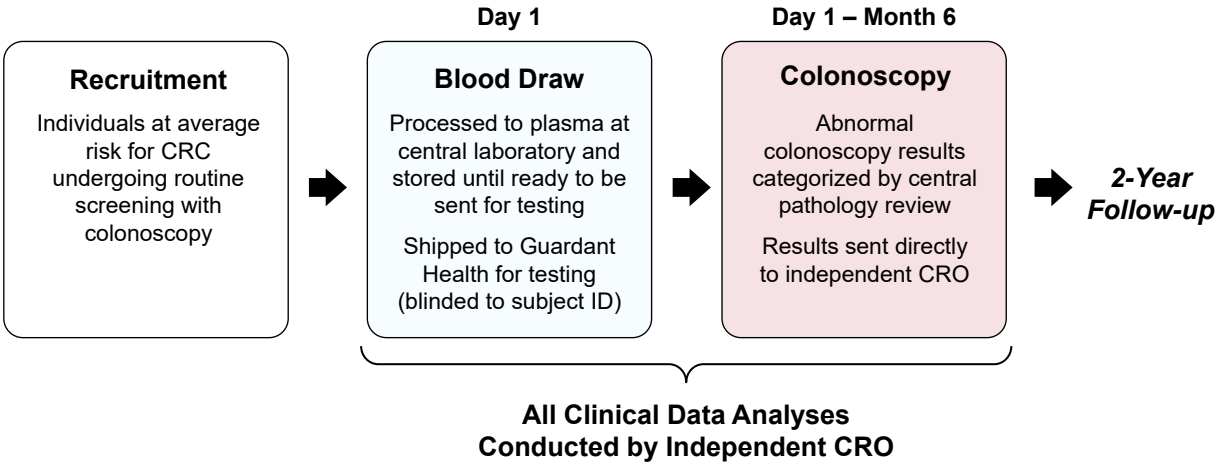
### *1.5.1 Study Design*

ECLIPSE was a prospective, single-arm study to collect blood samples to evaluate the performance of blood-based CRC screening tests (Figure 1). Participants, aged 45 to 84 years at average risk for CRC, undergoing routine screening colonoscopy, were enrolled from 265 sites across the US. Participants provided informed consent and underwent a study-related blood draw prior to any colonoscopy preparation. Participant results were not returned to participants or providers. The study results are based on the database as of March 2024.

Colonoscopy, performed per standard clinical practice, needed to have adequate bowel preparation and be within 183 days of the blood draw to be considered evaluable. All colonoscopy biopsy pathology reports were reviewed by central pathologists for consistent categorization as per protocol definitions:

- Category 1: Colorectal cancer, any stage
- Category 2: Advanced adenoma
- Category 3–5: Non-advanced adenoma (considered negative)
- Category 6: Negative

**Figure 1: ECLIPSE Study Design**




CRC=colorectal cancer; CRO=Contract Research Organization.

Advanced neoplasia (AN) was defined as CRC or AA (Category 1 or 2).

The two co-primary endpoints were:

- Sensitivity for CRC relative to colonoscopy (performance goal: lower bound of the 2-sided 95% confidence interval [CI] exceeds 65%), or 'CRC Sensitivity'
- Specificity for AN relative to colonoscopy (performance goal: lower bound of the 2-sided 95% CI exceeds 85%), or 'AN Specificity'

Secondary and key exploratory objectives included sensitivity for AA, positive and negative predictive value, and device performance by demographic and baseline characteristics.

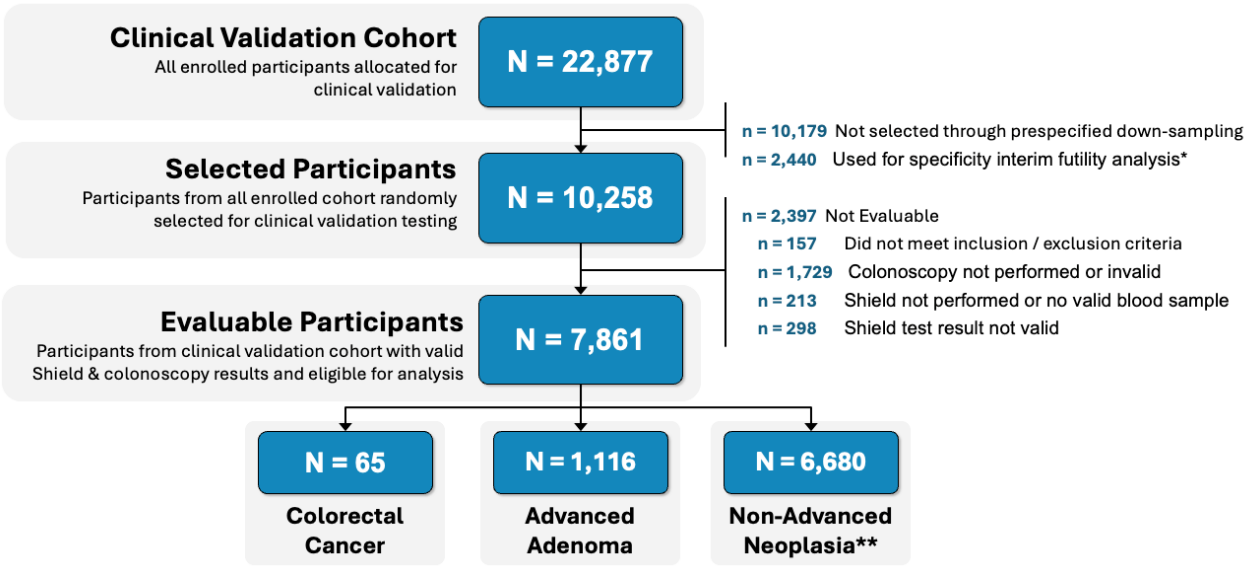
ECLIPSE was powered for the co-primary endpoints. Target enrollment was 68 evaluable participants positive for CRC on colonoscopy and 7,000 evaluable participants negative for AN on colonoscopy.

Age-stratified random down-sampling of the non-CRC participants was performed to match the US 2020 Census age distribution prior to sample testing.

### *1.5.2 Study Population*

Overall, 24,876 participants were enrolled across 265 clinical sites. A toral of 1,999 participants enrolled within a pre-specified time window were allocated to device development and not included in the clinical validation cohort (CV). From the 22,877 subjects allocated to the CV cohort, 12,698 participants were identified through age-stratified random down sampling of non-CRC participants and inclusion of all known CRC cases. From these participants, 2,440 were used for the Interim Specificity Analysis and were excluded from consideration for the primary analysis (Figure 2). The remaining 10,258 participants were the basis for the primary analysis. Of the evaluable 7,861 participants, 65 had CRC, 1,116 had AA, and 6,680 had non-AN (including normal colonoscopy) findings (Figure 2).

**Figure 2: ECLIPSE Participant Disposition**




*4 subjects in the interim futility analysis were determined to not meet I/E criteria
**Non-advanced adenomas, non-neoplastic findings, and negative colonoscopy

The average age of the 7,861 evaluable participants was 60 years and 53.7% were female. With respect to race in this evaluable cohort, 7.1% were Asian, 11.8% were Black or African American, and 78.5% were White; with respect to ethnic group, 13.3% were Hispanic or Latino. These demographics align with the intended use population in the US.

### *1.5.3 Results*

ECLIPSE met both co-primary endpoints set by Guardant: CRC sensitivity was 83.1% with a lower bound of the two sided 95% CI of 72.2%, which exceeded the pre-specified performance goal of 65%; AN specificity was 89.6%, with a lower bound of the two sided 95% CI of 88.8%, which exceeded the pre-specified performance goal of 85% (Table 1).

**Table 1: ECLIPSE Co-Primary Endpoint Results**

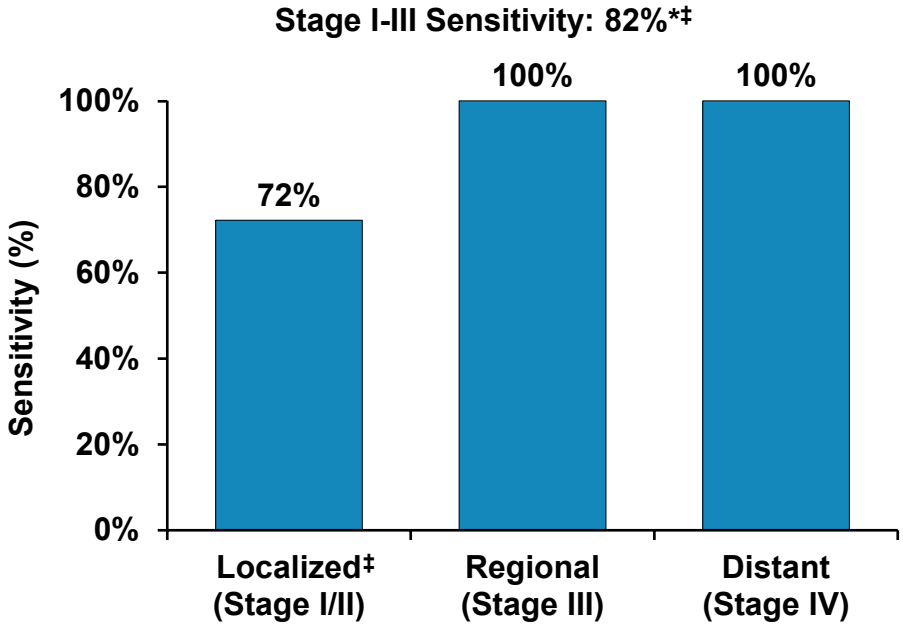
| | Colonoscopy/Histopathology | |
|---|---|---|
| **Shield Result** | **CRC (N=65)** | **Non-AN (N=6,680)** |
| Abnormal Signal Detected | 54 | 698 |
| Normal Signal Detected | 11 | 5,982 |
| Total | 65 | 6,680 |
| | CRC Sensitivity | AN Specificity |
| % (2-sided 95% Wilson CI) | 83.1 (72.2%–90.3%) | 89.6 (88.8%–90.3%) |

AN=advanced neoplasia; CI=confidence interval; CRC=colorectal cancer

There were no meaningful differences in CRC sensitivity relative to primary tumor location, tumor histologic grade, or demographic characteristics of the participants. CRC sensitivity by clinical Stage was determined:

- Stage I CRC: 54.5% (95% CI: 34.7%–73.1%; 12 of 22 cancers, including 5 'malignant polyps' that did not undergo full AJCC staging procedures)
- Stage II CRC: 100.0% (95% CI: 87.5%–100.0%; 14 of 14 cancers)
- Stage III CRC: 100.0% (95%CI: 82.4%–100.0%; 18 of 18 cancers)
- Stage IV CRC: 100.0% (95% CI: 70.1%–100.0%; 9 of 9 cancers)
- Combined Stage I, II (localized), and III (regional) CRC: 81.5% (95% CI: 69.2%–89.6%; 44 of 54 cancers) (Figure 3).
- Two individuals who did not undergo full AJCC staging and were lost to clinical follow up: 50.0% (1 of 2 cancers).

**Figure 3: CRC Sensitivity by Stage In ECLIPSE**




*Excludes 2 pathology confirmed, incompletely staged CRCs (sensitivity 1/2; 50%).
‡ Assumes 5 pathology confirmed, incompletely staged CRCs are clinical Stage I CRCs ("malignant polyps").
Source: Chung et al 2024.

AA sensitivity (secondary endpoint) was 13.2%. AA sensitivity trended higher in lesions of greater malignant potential based on size > 20 mm (17.2%) or presence of high-grade dysplasia (22.6%) or villous component > 25% (17.9%).

Using the CRC prevalence observed in the clinical validation cohort (0.41%), the positive predictive value of Shield for detection of CRC in this population was 3.0%. The positive predictive value for AN was 17.0% (95% CI: 15.0%–19.1%). The negative predictive value for CRC was 99.9% (95% CI: 99.9%–100.0%).

## 1.6 Real-world Adherence and Consideration of Public Health Outcomes

Currently available CRC screening tests have reported one-time adherence rates in the 28-68% range for FIT, 65-71% for mtsDNA, and below 50% for direct visualization procedures like colonoscopy and flexible sigmoidoscopy (Figure 13). A retrospective review of completion rates from the first 10,000 clinical orders for Shield (operated as a laboratory-developed test, LDT) demonstrated 96% adherence in screening age-eligible individuals. While this may be an overestimate skewed by early adopter bias, these data support that blood-based screening will yield higher adherence relative to current CRC screening modalities.

These adherence ranges, together with the published CRC sensitivity of respective screening modalities, can be used to estimate one-time CRC detection probability of CRC. As shown in Table 2, CRC detection for existing CRC screening modalities is meaningfully impacted by the one-time adherence for those tests. Even when assessing Shield at an adherence of 80%, which is significantly below the 96% observed with the LDT implementation, CRC detection remains at or above that of other CRC screening testing modalities (66%). This illustrates that the tests must be completed to be effective.

**Table 2: One-Time CRC Detection Rate for Available Screening Modalities Based on CRC Sensitivity and One-Time Adherence Described in the Literature**

| Screening Modality | CRC Sensitivity | One-Time Adherence | One-Time CRC Detection Probability (CRC Sensitivity x Adherence) |
|---|---|---|---|
| Colonoscopy | 95%[1] | 25–42%[5-9] | 24–40% |
| mtsDNA | 92–94%[2,3] | 65–71%[10-12] | 60–67% |
| FIT | 67–74%[2,3] | 28–68%[5-7, 13-19] | 19–50% |
| HSgFOBT | 50–75%[21,22] | 44–67%[8,23] | 22–50% |
| Shield | 83%[4] | 90–96%[20] | 75–80% |

CRC=colorectal cancer; FIT=fecal immunochemical test; mtsDNA=multi-target stool DNA; hsgFOBT=high-sensitivity guaiac-based fecal occult blood test

Sources: 1. Pickhardt et al 2011; 2. Imperiale et al 2014; 3. Imperiale et al 2024; 4. Chung et al 2024; 5. Quintero et al 2022; 6. Singal et al 2017; 7. Forsberg et al 2022; 8. Inadomi et al 2012; 9. Bretthauer et al 2022; 10. Conroy et al 2018; 11. Weiser et al 2020; 12. Miller-Wilson et al 2021; 13. Jensen et al 2016; 14.Olulluro et al 2016; 15. Binefa et al 2016; 16. Idigoras et al 2017; 17. Bretagne et al 2019; 18. Akram et al 2017; 19. Nielson et al 2019; 20. Raymond et al 2023; 21. Shapiro et al 2017; 22. Ahlquist et al 2008; 23. Fenton et al 2010

Screening strategies require repeat testing over time. Health outcomes models are used to estimate population level impact of screening beyond one-time CRC detection. Recently published models by CISNET have evaluated the impact of a blood-based test with CRC performance similar to that of FIT (74%, lower than that of Shield) against available screening strategies, including testing interval and test performance (van den Puttelaar et al 2024). The evaluation of clinical outcomes found that a blood test with screening participation of 80% results in CRC mortality reduction similar to stool-based testing with screening participation of 60%. This demonstrates the importance of

incorporating adherence in assessing public health outcomes. This finding is consistent with results from a public health outcomes model developed by Guardant Health that used adherence (rate of test ordered versus test completed) rather than screening strategy participation (Appendix 9.2).

In addition to direct comparison of one test strategy to another, it is relevant to consider the impact of introducing additional test modalities on the overall utilization of various screening options. A prior population-level study shows that the introduction of new non-invasive CRC screening options did not lead to a reduction in colonoscopy usage (Fisher et al 2021). A modeling analysis performed by Ladabaum et al evaluated the impact of introducing a blood-based test and the subsequent change on the test mix. In a comparison of current state (assuming 60% of the population is being screened, 40% with colonoscopy and 20% with other options) to the scenario where there is a 20% uptake of a blood-based test (10% in those currently unscreened, 10% in those who are currently screened with another test) with CRC performance similar to that of FIT (74% CRC sensitivity), this test mix yields CRC mortality reduction over the current state (Ladabaum et al 2024).

### *1.6.1 Direct and Indirect Risks*

The risks of Shield can be categorized as direct risks – those associated with the required blood draw – and indirect risks – the consequences of a false positive or false negative test result.

Shield presents a low direct risk; individuals are only required to undergo a routine blood draw, consistent with other blood-based diagnostic tests. There were no unanticipated adverse device effects across enrolled participants in ECLIPSE. Of the 43 adverse events (AEs) reported in ECLIPSE, 70% were minor discomfort related to phlebotomy and 30% were unrelated to study interventions.

Indirect risks are related to consequences of an individual receiving an inaccurate result, either a false positive or a false negative. First, a false positive could lead to an unnecessary colonoscopy and its associated risks; however, as colonoscopy is currently recommended in average-risk individuals, a false positive Shield result does not increase the current risk in the average-risk population. In fact, a true negative Shield result reduces the risk and burden of unnecessary colonoscopies in individuals, as a colonoscopy would have no benefit for these individuals. Second, a false negative Shield result could lead an individual with CRC to forgo diagnostic procedures such as colonoscopy. This risk is quantified in ECLIPSE (17%) and is within range of other non-invasive CRC screening tests (7–33%) (Chung et al 2024; Knudsen et al 2016). ECLIPSE demonstrated 100% sensitivity in Stage II, III, and IV disease, limiting the false negatives to Stage I and malignant polyps. Given the long sojourn time (time to clinical detection in the absence of screening) over years for CRC and the expected high adherence with Shield, this creates a potential time window to reduce the false negative impact.

## 1.7 Benefit-Risk Summary

Improving adherence to CRC screening in average-risk individuals is critical to reduce CRC-related mortality.

Shield increases CRC screening options. Shield is the first blood test with performance within range of other guideline recommended non-invasive testing options. The biggest expected benefit of a blood-based test like Shield would be improvement in CRC screening adherence, defined as the ability to complete CRC screening. Shield as a screening intervention can occur concurrently at a time that an individual interacts with the health care system for any health or wellness visit.

In a large study of individuals at average risk for CRC, ECLIPSE demonstrated CRC detection in range with other guideline recommended, non-invasive CRC screening tests. The indirect risks of false positives and false negatives are aligned with other non-invasive screening options. Advanced Adenoma detection is limited; however, given the multi-year transition timeframe for AA, this can allow multi-time point detection as demonstrated with longitudinal FIT testing, where multiple testing interventions yielded higher cumulative AA detection rates above a single point estimate performance (Randel et al 2021).

### *1.7.1 Benefit-Risk of Limited AA Detection*

Shield, like other non-invasive CRC screening tests, has limited ability to detect advanced adenomas relative to colonoscopy. Detection of these lesions can prevent the development of CRC, and thus impact CRC incidence and reduce mortality. CRC mortality reduction can also be achieved by detection of CRCs at an early asymptomatic stage while the disease is treatable. Shield's limited advanced adenoma detection could result in harm if the screening test is used only once in a lifetime. Given Shield's expected higher adherence (Table 13) and the extended dwell time of adenomas progressing to CRC, the risk of limited advanced adenoma detection and accrued harm would be reduced (as can be potentially supported by public health outcomes modeling, if all assumptions are appropriately vetted) (Forbes et al 2023). It is expected that Shield will improve overall population CRC screening rates, which outweighs the harm from individuals pursuing Shield over other CRC screening options.

### *1.7.2 Benefit-Risk Related to Diversion from Colonoscopy*

Like other non-invasive CRC screening tests, Shield has the potential to divert individuals from colonoscopy to lower-sensitivity tests. The incremental risk from approval of Shield is minimal as Shield has performance within range of currently available guideline-recommended, non-invasive CRC screening tests that are offered as primary screening tests. A label approving Shield to only be offered after declining or failing to complete other screening options is not appropriate as it would introduce a non-performance-based distinction between existing non-invasive screening tests that would result in loss of access to the benefits of blood-based testing.

Placing a restriction has been shown to hinder clinical adoption of new CRC screening tests and limit access for individuals who would benefit. Data consistently show that incorporating choices in CRC screening improves the rate of overall CRC screening completion (Inadomi et al 2012). The introduction of new CRC screening options has not been shown to reduce colonoscopy usage (Fisher et al 2021), and we expect Shield is similarly not likely to meaningfully change colonoscopy usage. Public health outcomes modeling shows that if some colonoscopy diversion occurs, it is unlikely to reduce the overall benefit of screening (Ladabaum et al 2024). Empowering physicians and individuals with multiple guideline-recommended CRC screening tests will increase the probability the individual will opt for the test they are most likely to complete instead of agreeing to an option that they later will not complete. The benefits of improved CRC screening completion rates by including Shield as a primary (first-line) CRC screening option alongside currently available guideline recommended stool-based tests outweigh any potential harms from individuals selecting Shield over other options at the population level.

### *1.7.3 Overall Benefit-Risk Profile for Shield*

Shield has CRC sensitivity and specificity within the range of other guideline recommended non-invasive primary CRC screening tests, including FIT, hsgFOBT, and mtsDNA and above that of current FDA approved blood-based test (Table 13, Figure 16). As such, it is appropriate for Shield to be approved for primary use for average risk individuals and the evidence demonstrates the clinical value of Shield in the proposed intended use. Incorporating Shield alongside the other guideline-recommended CRC screening options empowers physicians and their patients to complete CRC screening, bringing the benefit of selecting the most appropriate test for individuals and helping to achieve the 80% target set forth by the leading public health organizations, and reduce CRC mortality. Given the totality of evidence, this provides an additional and much needed opportunity to impact the second leading cause of cancer-related mortality – colorectal cancer. The performance of Shield as shown here demonstrates that the benefits of Shield as a primary CRC screening option outweigh the risks. Shield will fill an important gap in CRC screening options and has a favorable benefit-risk profile.

# 2 BACKGROUND ON COLORECTAL CANCER

**Summary**

- CRC is the fourth most diagnosed cancer and second leading cause of cancer-related death in the US, and disproportionately affects minority populations.
- The goal of CRC screening is the reduction in CRC-related mortality, which can be achieved through detection of early-stage CRC in asymptomatic individuals. As such, current guidelines recommend CRC screening for average-risk individuals who are 45–75 years old.
    - The 5-year survival rate for localized disease (Stage I/II) is 91%; the survival rate for metastatic disease (Stage IV) is only 14%.
    - An estimated 76% of CRC-related deaths occur in individuals who are not up to date with screening.
    - Adenoma detection and removal can prevent CRC development and reduce disease incidence.
- Approximately 58% of eligible individuals aged 45–75 years are up to date with CRC screening, well below the goal of 80% set by leading health organizations.
    - More than 50 million eligible American adults are unscreened for CRC.
    - The challenge with CRC screening is most participants do not actively refuse CRC screening, but rather they do not complete the prescribed CRC screening test, leading to delayed or deferred screening.
    - Differences in the proportion of individuals who are up to date with CRC screening by race/ethnicity, geographic region, age, and socioeconomic status underscore widespread access barriers with currently available screening options.
- Guideline-recommended CRC screening options inform clinical decision making and include both direct visualization (e.g., colonoscopy) and non-invasive stool-based tests; per the USPSTF, no screening test is recommended over another; choice of screening test should depend on individual preference and test availability.
- Including a non-invasive blood-based test as a screening option alongside existing screening options can address barriers to screening completion as the test can be completed as part of routine healthcare encounters.

## 2.1 Overview of Colorectal Cancer

CRC is the fourth most diagnosed cancer and second leading cause of cancer-related death in the US, with an estimated 53,010 deaths attributable to CRC in 2024 (Siegel et al 2024).

CRC primarily arises from a precursor lesion, the adenomatous polyp (i.e., adenoma), that grows from the epithelial cells of the colorectal mucosa. Adenomas that grow larger than 10 mm or have elements indicating a risk of malignant transformation (e.g., high-grade dysplasia or villous features) are defined as AAs (Fleming et al 2012). Left undetected and untreated, a proportion of AAs may continue to grow, develop additional features of dysplasia, and have the potential to eventually transform into carcinoma (Winawer 1999; Winawer et al 1992). Colorectal polyps, including both non-adenomas and adenomas, are common in the general population (Levy et al 2015). Approximately 30% of adults are thought to have colorectal polyps, and approximately two-thirds of these polypoid lesions are adenomas (Winawer 1999). The vast number of adenomas, even those with features classifying them as AAs, do not progress into a colorectal malignancy. Colonoscopy cannot always distinguish adenomas or advanced adenomas from other polyp histology; thus, polypectomy is routinely performed for lesions identified on endoscopy. The transition rate from adenoma onset to CRC development is estimated to be 12.5 to 25 years (Knudsen et al 2021). This slow transition from adenoma onset to CRC onset allows for multiple CRC screening opportunities over a lifetime, providing the ability to intervene along the disease development course and the potential to detect and remove adenomas, prevent colorectal cancer, and reduce CRC incidence and subsequently, disease mortality.

Once CRC has developed, tumor staging is consistent with other solid tumors and defined based on how far the cancer has spread within the body. In Stage 0 (carcinoma in situ), the cancer cells are only in the colorectal mucosa. In Stage I, the cancer has spread to the muscular layer of the colorectum but not to nearby tissue or lymph nodes. In Stage II, the cancer has grown through the wall of the colorectum and potentially to nearby tissues but has not spread to nearby lymph nodes. In Stage III, the cancer has spread to nearby lymph nodes but not to distant parts of the body. In Stage IV, the cancer has spread to one or more distant parts of the body (ACS 2024). The estimated time frame from CRC onset to a symptomatic diagnosis of CRC is estimated to be 4–5 years, in the absence of early detection through asymptomatic cancer screening (Knudsen et al 2021). Tumor size and location can influence the rate of transition through the stages of CRC. The 5-year survival rate for localized disease (Stage I-II) is 91%, and is 72% for regional disease (Stage III), while the 5-year survival for metastatic disease (Stage IV) is only 14% (Siegel et al 2024). These statistics highlight the ability to reduce CRC-related mortality by detection of early-stage (Stage I–III) disease where therapeutic intervention has the potential to result in a cure.

The risk of CRC increases with age, with the majority of cases and deaths occurring in individuals aged 65 years or older. While the incidence of CRC in Americans 65 years of age or older has decreased over the last decade, the incidence of CRC in younger Americans aged 55 years or younger has been increasing since the mid-1990s (Siegel et al 2024). CRC disproportionately affects minority populations, with American Indian/Alaska Native and Black/African American populations having the highest

incidence and mortality rates (Siegel et al 2024); this is further exacerbated by systemic barriers to current CRC screening options.

## 2.2 CRC Screening Guidelines and Goals

The multi-step process from normal colonic epithelia to adenomatous polyp to malignancy is a slowly progressive transition (Nguyen et al 2020), with only a small percentage of adenomas transitioning to malignancy, leading to a cancer diagnosis. The total dwell time, defined as time from adenoma onset to colorectal cancer diagnosis (in absence of screening intervention), has an estimated range of 17–29 years, with 12.5–25 years from adenoma onset to CRC onset and 4–5 years from CRC onset to symptomatic diagnosis (Knudsen et al 2021). This extended dwell time makes CRC an ideal public health target, and CRC screening is recommended for all adults beginning at age 45 years.

According to the World Health Organization, screening aims to identify apparently healthy people who are at higher risk of a health problem, such that early treatment or intervention can be offered to reduce mortality of the condition (World Health Organization 2020). In average-risk CRC screening, the goal is to reduce disease-related mortality by detecting cancer at an early stage, before metastatic cancer develops, where therapeutic intervention has the potential to result in a cure. Colorectal cancer screening is unique in that it also has the opportunity to prevent CRC through detection and treatment of advanced adenomas, thus reducing CRC incidence and mortality.

‘Average-risk’ individuals in the context of CRC screening are defined as those who do not have symptoms of CRC and do not have increased risk factors for the disease (i.e., prior diagnosis of CRC, adenomatous polyps, or inflammatory bowel disease; family history of CRC or known hereditary predisposition to CRC) (USPSTF et al 2021).

Asymptomatic screening of average risk individuals with any of the approved modalities significantly reduces CRC-related mortality through detection of early-stage disease, and is uniformly recommended by leading professional societies including the USPSTF, the US Multi-Society Task Force on Colorectal Cancer, and the American Cancer Society (ACS) for average-risk individuals who are 45–75 years old (ACS 2024; Levin et al 2008; Patel et al 2022; Rex et al 2017; USPSTF et al 2021; Wolf et al 2018). Clinical guidelines such as these are critical in helping physicians and their patients make the best evidence-based decisions about clinical care, and to understand how to incorporate existing CRC screening tests into practice (Guerra-Farfan et al 2023).

Guideline-recommended CRC screening options include both direct visualization tests (e.g., colonoscopy) and non-invasive stool-based tests. USPSTF evaluates the benefits, burden, and harms of the various CRC screening modalities compared with no screening. Screening recommendations are therefore based on a favorable benefit-to-harm ratio, timely screening intervals, and accessibility and accuracy of available screening tests.

Clinical outcomes modeling of direct visualization and stool-based screening tests show an average of 286–337 life-years gained, 42–61 CRC cases averted, and 24–28 CRC deaths averted per 1,000 average-risk individuals screened beginning at age 45, compared with no screening, depending on the screening method being used and assuming participants are fully adherent to screening recommendations over their lifetime (e.g., annual FIT screening or colonoscopy every 10 years) (Knudsen et al 2021; USPSTF et al 2021). Based on this evaluation, USPSTF does not recommend any one approved screening test over another; instead, guidelines state that eligible individuals can be screened by any recommended method, and choice of screening test should depend on individual preference and test availability (USPSTF et al 2021; Wolf et al 2018). Two recent studies performed independent outcomes modeling for a hypothetical blood-based CRC screening test that meets minimum performance metrics (74% sensitivity for CRC, 90% specificity for AN). Modeling results demonstrated that a test with this performance profile yields positive clinical outcomes as compared with no screening (Ladabaum et al 2024; van den Puttelaar et al 2024) and an improvement in CRC related deaths when blood-based testing is incorporated into the mix of screening options with some level of substitution of existing tests (Ladabaum et al 2024).

Decisions about preventive care, such as CRC screening, involve a partnership between clinicians and patients aimed at making informed decisions about healthcare interventions to maximize the likelihood that the patient completes the intervention. Clinical practice guidelines such as those set forth by the USPSTF and ACS provide evidence-based recommendations for providers, empowering them to engage in a discussion of the best test for each patient and the frequency at which the selected test should be repeated. This process, termed ‘Shared Decision Making’, is critical in the selection of the preferred CRC screening modality from the multiple current guideline-recommended CRC screening tests. The goal of this process is to maximize test adherence, defined as the likelihood individuals will complete the prescribed CRC screening test. As often stated in the context of CRC screening, “The best screening test is the one that gets completed by the patient” (Carethers 2024).

## 2.3 Current Colorectal Cancer Screening Options

Estimates of current CRC screening test usage in the group of individuals are up to date with screening have identified four primary modes of CRC screening: colonoscopy (60.3%), FIT (fecal immunochemical testing, 18.3%), mtsDNA (multi-target stool DNA, 14.2%), and high-sensitivity guaiac FOBT (fecal occult blood testing, 6.6%) (Fisher et al 2021). All 4 of these testing modalities are recommended by the USPSTF (Lin et al 2021). Only two CRC screening options, guaiac FOBT (gFOBT) and flexible sigmoidoscopy, have data from randomized clinical trials demonstrating a decrease in CRC-related mortality (USPSTF et al 2021). CRC screening experts have therefore assumed that a test with similar or better test performance metrics will yield similar or higher reductions in CRC incidence and mortality reduction (Zauber 2015). In fact, gFOBT is no longer commonly used and has generally been replaced with

high-sensitivity gFOBT (hsgFOBT), which is currently a USPSTF-recommended screening option (USPSTF et al 2021).

Colonoscopy is unique amongst screening modalities in its ability to visualize, biopsy, and resect suspected premalignant and malignant lesions (e.g., adenomas). Colonoscopy is the most established and accurate screening modality (Table 3). For those who undergo a screening colonoscopy that does not yield any findings, the recommended interval for repeat screening is 10 years. Screening by colonoscopy results in one of the highest estimated reductions in CRC mortality, as it includes both detection of early-stage, asymptomatic cancers and removal of adenomas. However, the impact of colonoscopy is dependent on the quality of the procedure. Data suggest significant variability in colonoscopy quality, which in turn impacts the effectiveness of the colonoscopy to visualize and treat lesions in a meaningful way (Lieberman et al 2007). Several colonoscopy quality indicators, such as withdrawal time, completeness of bowel preparation, and adenoma detection rate of the endoscopist, are continuously monitored to assess the quality of an endoscopist and/or a screening program. Unsatisfactory measures of any of these or other indicators have been shown to be associated with an increased risk of missed cancers and/or pre-cancerous lesions, negatively impacting the effectiveness of colonoscopy.

While colonoscopy is the most accurate CRC screening test, it is also considered the primary source of screening harm, both as a screening test and as a follow-up diagnostic procedure following a positive non-invasive stool-based test. While fatal complications are extremely rare, known non-fatal complications can affect quality of life. The main colonoscopy complications are serious bleeding events and colonic perforations (USPSTF et al 2021). In a population undergoing screening colonoscopy, the risks of colon perforation and major bleeding were 3.3 per 10,000 (95% CI: 2.2–4.3) and 14.9 per 10,000 (95% CI: 9.0–20.8), respectively, and increase with age. Much of the accrued harm comes from the need to remove all observed lesions given the inability to differentiate pre-cancerous versus benign lesions.

The trade-off between increasing detection and intervention of polyps (and presumably catching AA) to reduce the relatively low prevalent CRC needs to consider the risks of such an approach. As stated in Kalager, et al “Consequently, although endoscopic polyp removal is far less invasive and has smaller risks on a population level, the accumulated risk and burden is not negligible, and needs to be taken into account in the policy making in relation to screening programs. Further, in the United States, the rates of surgical removal of precancerous polyps are increasing, and 1 in 7 patients experienced a major postoperative event. Thus, overtreatment of polyps may have more severe consequences than previously anticipated " (Kalager et al 2018). With low transition rate of adenomas the majority of procedures do not provide the intended benefit and procedural risk becomes a greater issue. In essence, the harm of over-diagnosis and over treatment using colonoscopy screening is often not reflected in screening outcomes when assessing CRC incidence or mortality reduction (Kalager et al 2018)

Further, of the available screening options, colonoscopy has the lowest adherence for average-risk individuals. Estimates of adherence with colonoscopy in a screen-eligible population range from 25–42% (Bretthauer et al 2022; Forsberg et al 2022; Inadomi et al 2012; Quintero et al 2012; Singal et al 2017). A recent study found the effectiveness of screening colonoscopy in preventing CRC-related death was significantly decreased by the suboptimal adherence rate to colonoscopy (42%) of the population under study (Bretthauer et al 2022).

Stool-based CRC screening tests detect blood and other biomarkers indicative of CRC from individuals' feces. Several stool-based tests are recommended by the USPSTF, including high-sensitivity guaiac fecal occult blood test (hsgFOBT), FIT, and mtsDNA. Stool-based tests may be a patient-preferred screening option, given the convenience of at-home testing and the lack of a requirement for bowel preparation; however, the tests' performance, in terms of the ability to accurately detect CRC, is lower than that of colonoscopy (Table 3) (Imperiale et al 2024; USPSTF et al 2021). Individuals who have a positive stool-based test warrant further investigation through colonoscopy.

Given stool-based tests' performance, clinical guideline committees recommend a more frequent testing interval than for colonoscopy - every year for FOBT and FIT, and every 1 to 3 years for mtsDNA. The primary harms for stool-based tests come from false positive and false negative screening results, or from follow-up diagnostic colonoscopy after positive stool-based screening results (USPSTF et al 2021). Adherence to stool-based screening tests, meaning the completion rate of the prescribed tests by participants, is estimated to be upwards of 20% higher than adherence to colonoscopy, ranging from 28–68% (Akram et al 2017; Conroy 2018; Lin et al 2021).

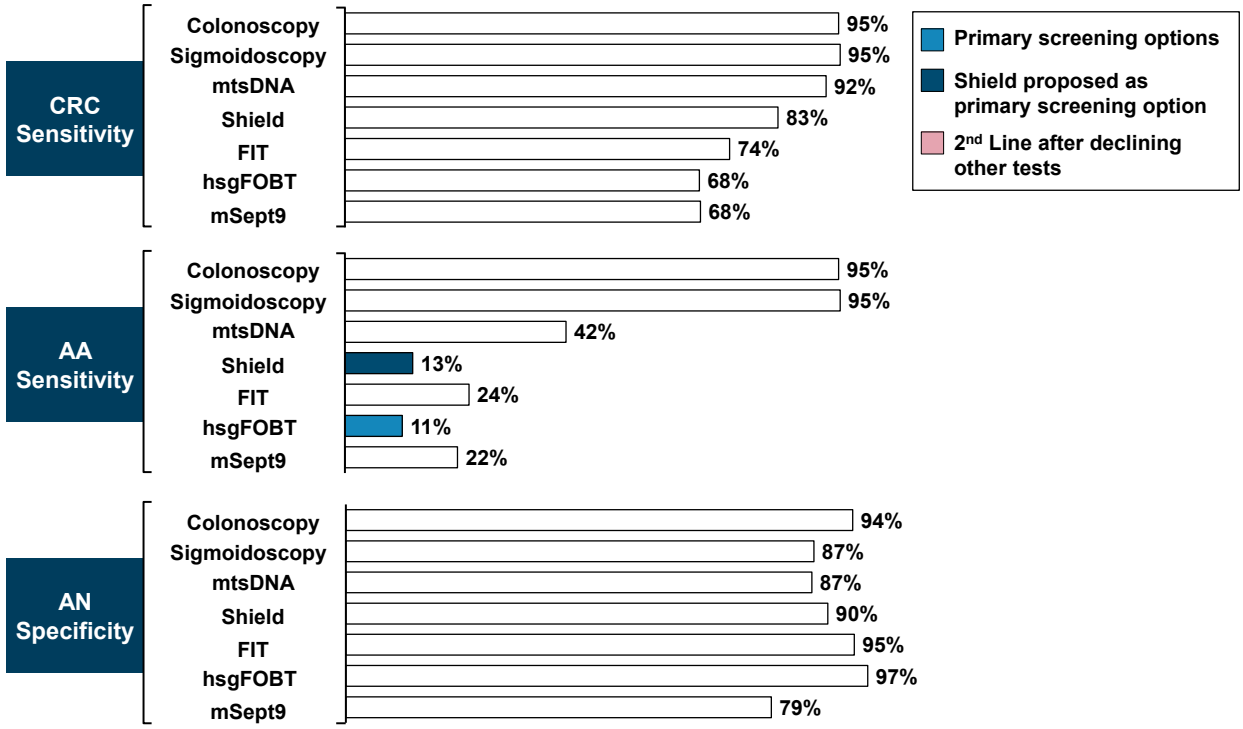
The FDA has approved a blood-based CRC screening test, Epi proColon, which detects methylated Septin 9 (mSept9) DNA from blood (PMA P130001). This test had specificity (79%) much lower than that of stool-based testing (Table 3 and Figure 4). As such, the FDA approved this test for use only in those who have declined all guideline-recommended CRC screening (termed "second-line"), and it is not recommended as a screening test in any clinical guidelines. The second-line label and the test performance limited patient access to the test and significantly hindered clinical adoption.

## Table 3: Performance of the CRC Screening Tests

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| | 94.7%[1] | 92.3%[3] | 93.9%[4] | 73.8%[3]<br>67.3[4] | 68%[2] | 95.0%[8+] | 68.2%[6] |
| | 89.1-94.7%<br>(≥ 10 mm adenomas)[2,9] | 42.4%[3*] | 43.4%[4**] | 23.8%[3*]<br>23.3[4**] | 11%[2]<br>(≥ 10 mm adenomas) | 95.0%<br>(≥ 10 mm adenomas)[8+] | 21.6%[6***] |
| | 94.2%<br>(≥ 6 mm adenomas)[2,9] | 86.6%[3]<br>(AN) | 90.6%[4]<br>(AN) | 94.9%[3]<br>94.8[4] (AN) | 97%[2]<br>(AN) | 87.0%[8] | 79.1[6] (AN) |

Note: Not head-to-head trials
#Computed tomography colonography is a guideline recommended screening strategy not included due to low usage.[5]
+By assumption, per CISNET
†Currently not yet commercialized[10]
‡Discontinued marketing[11]
*Includes AA (HGD or ≥ 25% villous histologic features or ≥ 10 mm) and sessile serrated polyps ≥ 10 mm
**Includes adenomas or sessile serrated lesions measuring ≤10 mm, lesions with villous histologic features, and HGD
***Includes polyps ≥ 10 mm, polyps with HGD, or villous components.
AA=advanced adenoma; AN=advanced neoplasia; CRC=colorectal cancer; FIT=fecal immunochemical test; hsgFOBT=high sensitivity guaiac fecal occult blood test; HGD=high-grade dysplasia; mSept9=methylated Septin 9; mtsDNA=multi-target stool DNA.
Sources: 1. Pickhardt et al 2011; 2. Lin et al 2021; 3. Imperiale et al 2014; 4. Imperiale et al 2024; 5. Fisher et al 2021; 6. Potter et al 2014; 8. Knudsen et al 2021; 9. Lin et al 2016; 10. Exact Sciences 2024; 11. Epigenomics 2023.

## Figure 4: Graphical Depiction of Performance of CRC Screening Tests

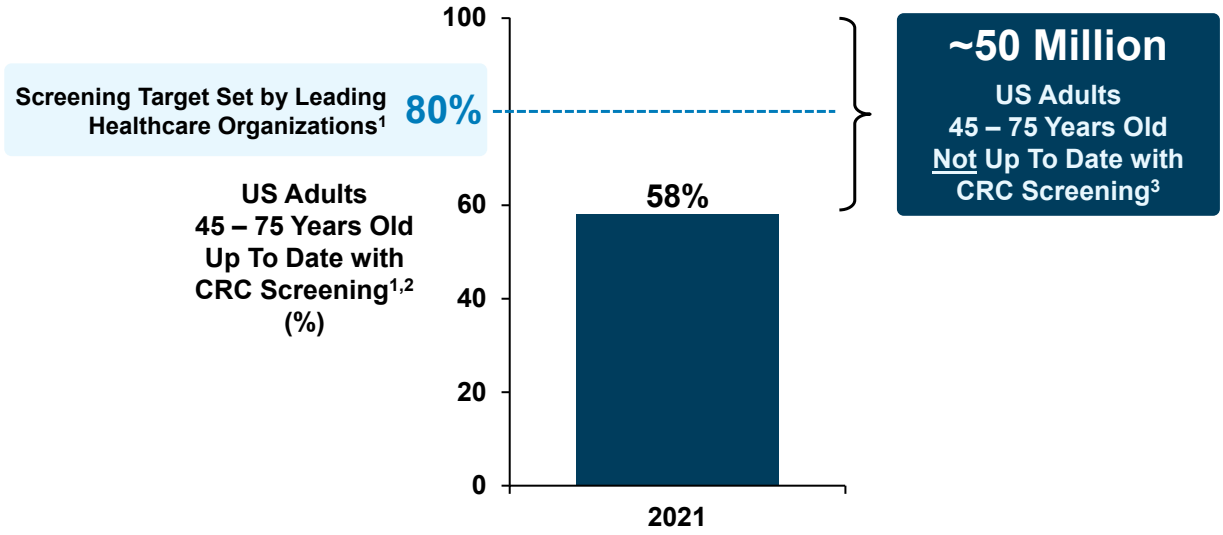



AA=advanced adenoma; CRC=colorectal cancer; FIT=fecal immunochemical test; hsgFOBT=high sensitivity guaiac fecal occult blood test; mSept9=methylated Septin 9; mtsDNA=multi-target stool DNA.
Note: 2nd line defined as after declining guideline-recommended primary screening tests

## 2.4 Colorectal Cancer Screening Rates

The known positive overall health impact of asymptomatic CRC screening led the National Colorectal Cancer Roundtable to set forth a target goal for 80% of screening-eligible individuals to be up to date with screening recommendations, in every community (Wender et al 2020). Achieving this screening rate would lead to a significant decrease in CRC-related deaths. Despite the availability of multiple screening options, the proportion of eligible individuals who are up to date with CRC screening recommendations has remained below the target goal of 80% (Figure 5). Individuals who are not up to date with screening are at risk of having undetected and untreated CRC; an estimated 76% of CRC-related deaths occur in individuals who are not up to date with screening (Doubeni et al 2019). CRC is still the second leading cause of cancer-related death in the US.

**Figure 5: Percentage of Adults Aged 45–75 Years Who Received CRC Screening**




CRC=colorectal cancer
Sources: 1. Wender et al 2020; 2. Siegel et al 2023; 3. US Census Bureau 2023.

The US remains far short of this 80% goal, with only 58% of individuals aged 45–75 years up to date with CRC screening recommendations (Siegel et al 2023), meaning that approximately 50 million screen-eligible Americans are not up to date with CRC screening and are at risk for CRC death (Figure 5) (Siegel et al 2023; US Census Bureau 2023b). This is despite the introduction of new stool-based tests with improved performance metrics (i.e., mtsDNA) available now for over a decade, numerous national and local education campaigns (e.g., March is Colorectal Cancer Awareness Month), and federal legislation implemented to reduce cost sharing for individuals undergoing diagnostic colonoscopies following a positive non-invasive CRC screening test.

Individual preferences and values are important considerations and can form barriers to CRC screening completion and contribute to low rates of CRC screening. These factors

include time required to perform screening, scheduling challenges, concern over test invasiveness and pain, fear of the test, discomfort or embarrassment associated with endoscopic examinations, distance from the test provider, provider capacity, and lack of physician recommendation for screening (Seeff et al 2004). The majority of individuals understand the importance of CRC screening and have been educated on the benefit of early cancer detection. The challenge with CRC screening is that some participants do not actively refuse CRC screening during a healthcare encounter (Keogh et al 2017). Rather, the prescribed tests do not get completed, as current CRC screening options are perceived as burdensome, leading individuals to delay or defer screening. For example, wait times for screening colonoscopy can extend upwards of 3-6 months (Hubers et al 2020). Reasons for not completing stool-based testing after initially agreeing include “forgetfulness” and “procrastination,” as well as concerns with the multi-step process (Schneider et al 2023). Tracking and monitoring CRC screening completion is challenging to manage within the primary care setting, requires institutional infrastructure, and is often infeasible. Oftentimes, providers are not aware that the individual did not follow through with the agreed upon CRC screening test until the next health care encounter. This reality highlights the barriers to test access that requiring participant refusal (a second-line label) presents, as illustrated by the experience with the Epi proColon test.

Within the overall population of screen-eligible individuals, there are large differences in the proportion of individuals who are up to date with CRC screening recommendations by race and ethnicity, geographic region, age, and socioeconomic status, which underscore the widespread access barriers with currently available CRC screening options. Hispanic individuals have a lower screening rate compared with Non-Hispanic White individuals (52% vs 61%), and Asian and American Indian/Alaska Native populations have the lowest screening rates by race (50% and 52%, respectively) (Siegel et al 2023).

These statistics highlight the urgent unmet need for new patient-preferred CRC screening options that individuals will complete. Increasing the proportion of individuals who are up to date with CRC screening can be achieved by expanding the types of CRC screening test options available, so that it becomes easier to encourage population wide use (Carethers 2024). In fact, data show that CRC screening completion rates are higher when an individual is provided choices in their test options (Inadomi et al 2012; Lin et al 2021; Steffen et al 2014). Incorporating a blood-based test, performed as part of any health care encounter, would provide an additional screening option thus improving screening rates (Adler et al 2014; Liang et al 2023).

### 2.5 Unmet Medical Need

Suboptimal adherence and barriers to screening with current modalities point to a pressing need for additional non-invasive CRC screening options. A blood-based test with performance similar to currently available non-invasive screening modalities will improve adherence to screening and facilitate screening for populations who might not

otherwise comply with existing screening modalities (Coronado et al 2024; Schneider et al 2023).

Blood-based screening tests can improve the capacity of the diagnostic pathway and are more likely to be completed than stool-based tests (Liles et al 2017; Maringe et al 2020). Additionally, colonoscopy screening alternatives can aid in triage of the invasive colonoscopy procedure to those with the highest likelihood of disease, further reducing screening-related harm.

Incorporating a non-invasive blood-based CRC screening test alongside other screening options can address barriers to screening such as ease of completion and accessibility, given that it can be drawn and completed as part of routine and opportunistic health care encounters, and offers convenience with no special preparation, dietary changes, sedation, or additional time off work required (Rich et al 2020) and ensure more preventable CRC deaths are avoided by eligible individuals being adherent to this well-established intervention.

# 3 CFDNA AS A BIOMARKER FOR COLORECTAL CANCER

**Summary**

- Tumor-derived cfDNA fragments carry genomic sequence mutations and epigenomic modifications present in the tumor into the bloodstream, offering opportunities for non-invasive CRC screening.
- cfDNA is used in FDA-approved companion diagnostic tests, including Guardant360 CDx, for therapy selection in patients with advanced cancer.
- Shield analyzes cfDNA for 3 types of biomarkers (somatic mutations, cfDNA fragmentation patterns, and aberrant methylation) across > 2,000 genomic regions to detect the presence of CRC.

Existing integration of peripheral blood analysis in the US healthcare system and high adherence to these types of tests makes blood-based testing an attractive approach to improving CRC screening rates. One of the most promising approaches to detect the presence of tumor is via the detection of tumor-derived DNA in the bloodstream.

## 3.1 Overview of cfDNA

During cellular death (e.g., apoptosis, necrosis), DNA is released from the cell into the circulatory system, including the bloodstream. This cfDNA is digested into smaller fragments which are found in the plasma component of blood. cfDNA fragments carry the underlying genomic sequence mutations and epigenomic modifications of the original cell. Since colorectal tumors are known to have a large burden of both genomic and epigenomic alterations (Cancer Genome Atlas Network 2012; Lao and Grady 2011), tumor-derived cfDNA provides the opportunity to identify tumor presence by analyzing cfDNA accessible from a simple blood draw (Stroun et al 1989).

## 3.2 Use of cfDNA in Cancer Detection

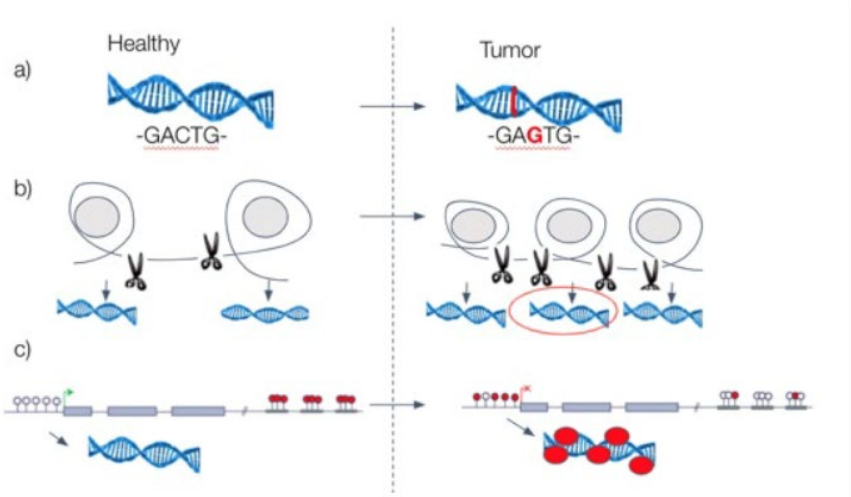
Analysis of cfDNA has been successfully used in companion diagnostic and tumor profiling in vitro diagnostics as a non-invasive tool for therapy selection in advanced cancer, including an FDA-approved test from the Sponsor (PMA P200010). cfDNA profiling is also included in the National Comprehensive Cancer Network (NCCN) guidelines for 17 different cancers, including CRC, non-small-cell lung cancer, and breast cancer (National Comprehensive Cancer Network (NCCN) 2024a; National Comprehensive Cancer Network (NCCN) 2024b; National Comprehensive Cancer Network (NCCN) 2024c).

Compared to the clinical applications in advanced-stage disease, where the tumor burden in circulation is generally higher, detecting the presence of cancer in asymptomatic average-risk individuals presents a unique challenge due to the lower levels of the fraction of the tumor-derived cfDNA in plasma (tumor fraction). In

individuals with colorectal neoplasia, tumor fraction is dependent on the disease stage and the lesion size, among other factors. While pre-cancerous colorectal lesions, e.g. advanced adenomas, contain both somatic mutations and aberrant methylation, the amount of shedding is lower due to multiple biological factors (Widman et al 2022). A background level of non-tumor-derived cfDNA molecules is also present in plasma due to the normal turnover of blood cells and healthy tissues (Loyfer et al 2023), and tumor-derived cfDNA needs to be distinguished from the background for cancer to be detected accurately.

Tumor-derived cfDNA can be identified using either genomic or epigenomic alterations. Genomic alterations refer to the changes in the genomic sequence of the tumor, while epigenomic alterations encompass changes that do not alter the genomic sequence but alter instead how the DNA is transcribed and used functionally by cells. Typical epigenomic alterations measured in cfDNA include aberrant methylation (Hanley et al 2017) and changes in fragmentation patterns (Ding and Lo 2022). These changes are illustrated in Figure 6. For example, tumor-derived cfDNA molecules from a specific genomic location may contain somatic mutations, which distinguish them from healthy cell-derived cfDNA molecules (top panel, a). cfDNA fragmentation patterns (middle panel, b) do not alter the underlying sequence but change where the cfDNA is fragmented when it reaches the circulation. Finally, methylation alterations (bottom panel, c) also do not alter the underlying sequence but represent chemical modifications of the DNA that can be directly measured. Methylation alterations provide strong tumor detection capability because aberrant methylation is an early marker of colorectal cancer and occurs consistently across CRC tumors in different individuals at many distinct genomic regions (Hanley et al 2017; Simmer et al 2012). The Shield test measures all 3 features shown in Figure 6 to detect tumor-derived cfDNA.

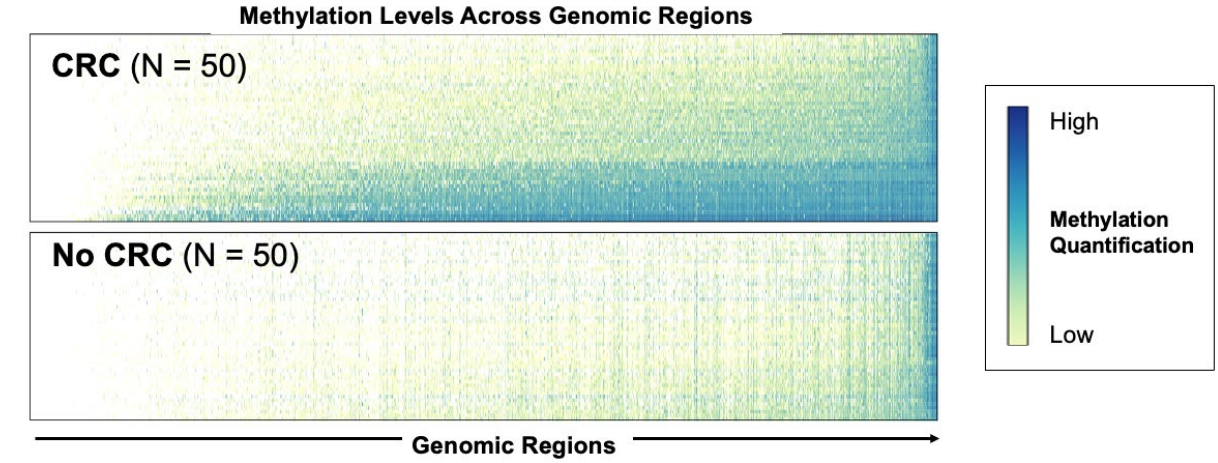
**Figure 6: cfDNA Biomarkers Differentiating Healthy and Tumor Tissue**




cfDNA=cell-free DNA
a) Somatic variants; b) cfDNA fragmentation pattern changes; c) Methylation changes

To effectively detect tumor-derived cfDNA in asymptomatic CRC in average-risk individuals, Shield measures aberrant methylation across more than 2,000 genomic regions. As an example, Figure 7 shows the differential methylation patterns observed between CRC and healthy individuals' cfDNA in plasma across differentially methylated genomic regions targeted by the Shield test. In this figure, genomic regions are shown along the horizontal axis, and individual cases and controls are shown along the vertical axis across a cohort of participants with CRC of all stages (top) and healthy donors (bottom). At the selected genomic regions, observed methylation levels in the CRC samples are higher than in the healthy samples (as denoted by darker colors in the heatmap). This example illustrates the ability of the Shield panel of methylation biomarkers to discriminate CRC-positive individuals from healthy individuals using many unique regions in the genome to ensure detection of CRC even in samples with low tumor fraction in circulation.

**Figure 7: Methylation Patterns in CRC Versus Healthy Individuals**




CRC=colorectal cancer

# 4 OVERVIEW OF SHIELD TEST

**Summary**

- Shield is a qualitative in vitro diagnostic test intended for CRC screening in individuals at average risk of the disease and aged ≥ 45 years.
- Shield includes a Blood Collection Kit (BCK), reagents, instruments, and software for sample testing, data analysis, and report generation; sample testing and analysis are conducted in a Guardant Health laboratory.
- Shield classification models were developed and locked based on independent development cohorts of more than 3,500 samples and independently verified prior to validation.
- The Shield classification threshold specificity target was set at 90% based on clinical benefit-risk considerations.

## 4.1 Intended Use / Indications for Use

The Shield test is a qualitative in vitro diagnostic test intended to detect colorectal cancer derived alterations in cell-free DNA from blood collected in the Guardant Shield Blood Collection Kit.

Shield is indicated for colorectal screening in individuals at average risk of the disease, age 45 years or older. Patients with an “Abnormal Signal Detected” may have colorectal cancer or advanced adenomas and should be referred for colonoscopy evaluation. Shield is not a replacement for diagnostic colonoscopy or for surveillance colonoscopy in high-risk individuals. The test is performed at Guardant Health, Inc.

## 4.2 Device Overview

Shield is designed as a high throughput automated test performed in the CLIA certified, CAP accredited, New York State Department of Health approved Guardant Health Clinical Laboratory. Shield includes a BCK that is distributed for blood collection and then shipped to Guardant Health, reagents for sample testing, laboratory instrumentation with customized automated methods to automate the workflow, and software for sample and reagent tracking, data analysis, and report generation.

The BCK comprises all components used in the collection, stabilization, packaging, and transportation of whole blood samples and is the only test component intended for external distribution. The kit contains 4 vacuum-sealed 10 ml Streck blood collection tubes (BCTs) and packaging material with instructions for kit storage, sample collection, and shipping after samples are collected.

Once the BCK is shipped and received at Guardant Health, the Shield sample processing workflow (Figure 8) comprises 3 stages: wet lab **sample testing** to generate

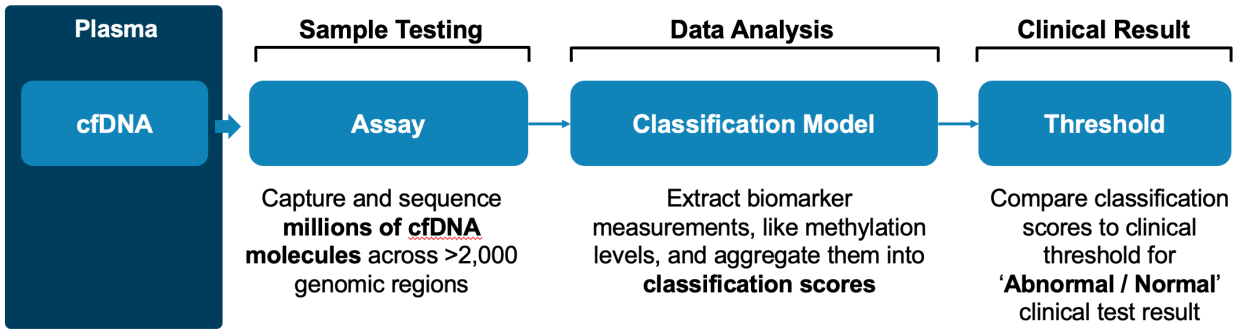
sequencing data for informative cfDNA molecules, **data analysis** to generate classification scores, and **clinical result** generation to convert classification scores into a simple Abnormal/Normal sample-level CRC detection result.

Sample testing starts from processing whole blood samples through plasma isolation and cfDNA extraction. Shield reagents and automation methods are then used to capture, amplify, and sequence millions of individual cfDNA molecules across more than 2,000 informative genomic regions. This process generally takes 5–7 days to complete.

Sample data analysis starts from the sequencing data and uses custom bioinformatics pipelines to extract relevant biomarker measurements, including tumor-derived somatic mutations, fragmentation patterns, and cfDNA methylation levels across relevant genomic regions. The Shield Classification Model then aggregates measurements associated with individual biomarkers to generate 2 quantitative classification scores which indicate the likelihood of tumor presence based on the biomarkers extracted from the sequencing data.

The result generation step compares 2 sample-level classification scores to their respective predefined clinical decision thresholds (referred to as simply 'thresholds' below). The Shield test returns the result of "Abnormal Signal Detected" when either of the 2 classification scores exceed their respective thresholds, and "Normal Signal Detected" otherwise.

**Figure 8: Shield Sample Processing Workflow**




cfDNA=cell-free DNA

The Shield assay originally included an independently assessed protein component. While both cfDNA-only and combined configurations were assessed in ECLIPSE, only the cfDNA-only configuration is being reviewed by the agency.

## 4.3 Shield Classification Model

The two quantitative classification scores used to generate the final Shield test outcome are referred to as the Methylation Regression Score (MR Score) and the Integrated Score. The MR Score is the quantitative score from a linear model for quantifying the fraction of tumor-derived cfDNA molecules in a sample based on the observed molecule

counts in the differentially methylated regions. The Integrated Score is the quantitative score from a logistic regression model developed to detect tumor presence based on the joint assessment of aberrant methylation signals, fragmentation patterns and a qualitative mutation detection status. Hereafter, MR Score and Integrated Score are collectively referred to as “classification scores.”

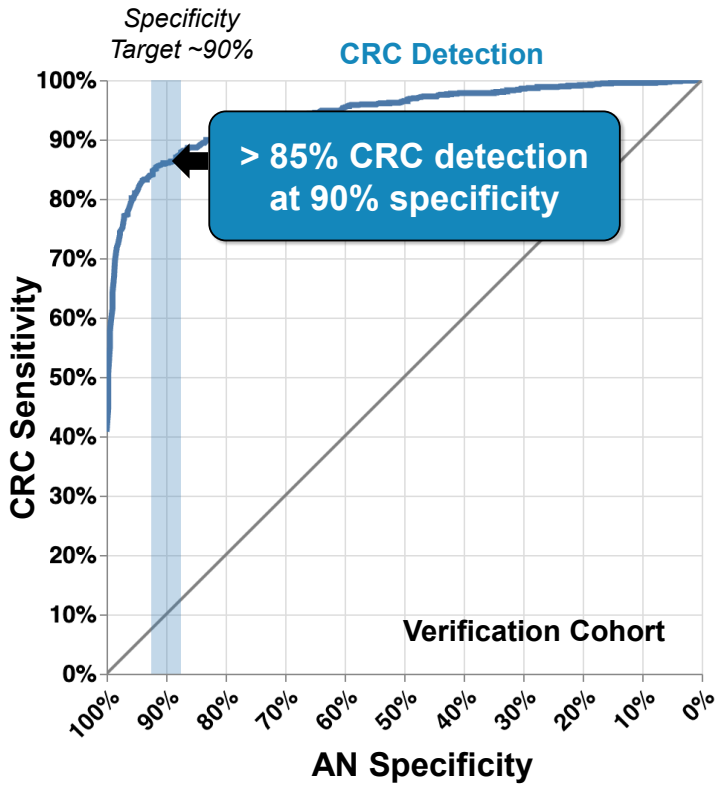
### 4.4 Development of the Shield Classification Model

The details of classification development have not been fully reviewed by the FDA. Efficient capture of the informative cfDNA molecules by the assay during sample testing creates the basis for CRC detection using appropriate analysis methods and classification models to extract the signals differentiating CRC and normal samples. During development, the cfDNA classification models and their parameters were optimized based on large training datasets that were designed to represent typical variation in biomarkers observed for representative CRC cases and colonoscopy-confirmed negative controls. In Shield development, all classification models were trained and locked before clinical validation sample testing was initiated, ensuring independence of performance estimates.

Shield classification models were trained based on independent training cohorts of 1,470 known CRC cases representative of all cancer stages and 2,340 cancer-free controls. To ensure robust model specification, these training samples were tested using different instruments, reagent lots, operators, and at different time points such that this training dataset represented both biological and technical sources of variability.

Prior to clinical validation, the performance of the Shield test was verified utilizing an independent verification cohort of samples including 1,050 known CRC cases across all cancer stages and 710 colonoscopy-confirmed controls without AN. In this verification cohort, sensitivity for CRC detection against the specificity for normal controls without AN (Figure 9) demonstrated the strong detection capability of the device. The area under the curve (AUC) was 0.94, indicating that the probability that a randomly selected CRC case has a Shield test value greater than a randomly selected non-AN control was 0.94. In the range of the performance target of 90% AN specificity, CRC sensitivity exceeded 85%, meeting the prospectively established design specifications.

**Figure 9: CRC Sensitivity Versus AN Specificity for Shield Verification Cohort**




AN=advanced Neoplasia, defined as CRC or advanced adenoma; CRC=colorectal cancer.

### *4.5* Shield Classification Threshold

The output of the Shield Classification Model is a combination of two classification scores (numerical values corresponding to MR score and Integrated Score) that represent the strength of the tumor-associated signal in a particular sample. These two classification scores are translated to a clinically meaningful “abnormal/normal” result by comparing their values to their respective clinical decision threshold values. If either of these two classification scores exceed their respective thresholds, the test result is ’Abnormal’. Otherwise, the test result is ‘Normal’. These two threshold values were established during development to meet the prespecified performance target.

Based on the clinical benefit-risk considerations for a blood-based CRC screening test, where individuals with positive results would be referred for diagnostic colonoscopy evaluation, this performance target was set at a specificity of 90% for individuals with negative colonoscopies during Shield development based on the specificity performance target defined in National Coverage Analysis CAG-00454N from the Centers for Medicare & Medicaid Services.

### 4.6 Analytical Validation

Guardant Health conducted analytical validation studies to establish analytical performance characteristics and demonstrate that the Shield assay is suitable for its

intended purpose. The suite of analytical studies and their designs were aligned to relevant FDA and Clinical & Laboratory Standards Institute (CLSI) guidelines and Agency interactions and involved a cumulative total of > 15,000 sample evaluations.

Studies included evaluation of limit of blank, limit of detection, assay precision (within-lab reproducibility and precision profile simulations), analytical accuracy, analytical specificity (endogenous and exogenous interfering substances), cross-contamination and carry-over, reagent lot-to-lot interchangeability, assay tolerance to variation in the critical steps of the assay workflow (guardbanding), cfDNA input guardbanding, and reagent and sample stability.

The studies followed predefined testing protocols and performance objectives, and all study objectives were met.

# 5 ECLIPSE STUDY

**Summary**

- ECLIPSE is a prospective, multicenter study designed to evaluate the performance of Shield to detect CRC in average-risk individuals.
    - Baseline demographics were representative of the intended use population.
- Both co-primary endpoints in ECLIPSE were met, demonstrating CRC sensitivity of 83% and AN specificity of 90% of Shield compared with colonoscopy diagnosis.
    - Sensitivity performance was consistent across baseline demographics subgroups.
    - Sensitivity in localized (Stage I/II) CRC was 72% and was 100% in regional (Stage III) and metastatic (Stage IV) CRC.
    - Sensitivity for AA was 13% and trended higher for high-grade dysplasia (23%) and lesions above 20mm in size (17%).
- Shield presents low risk for adverse device effects on blood draw. The potential for inaccurate results when balanced with the potential for increased screening adherence and repeated testing with a blood-based screening option helps to mitigate indirect risks.

## 5.1 Study Design

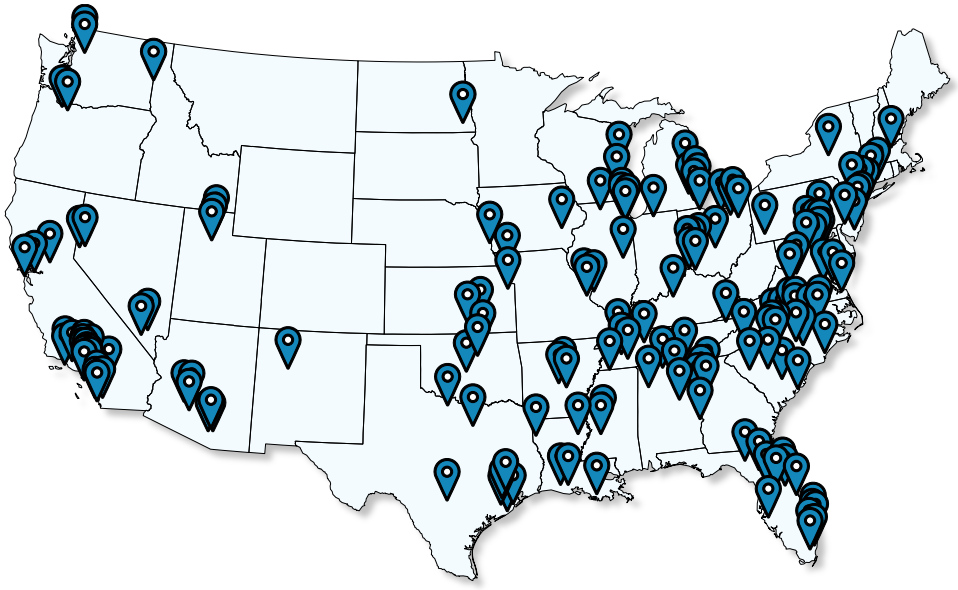
ECLIPSE was designed as a prospective, single-arm study to collect relevant samples and evaluate the performance characteristics of blood-based CRC screening test relative to colonoscopy in participants aged 45 to 84 years at average risk for CRC.

Participants presenting for CRC screening at 265 sites in the US, including 20 academic (or VA) sites and 245 community sites (Figure 10). Enrolled participants underwent a study-related blood draw prior to colonoscopy preparation. Blood samples were drawn, shipped to a central independent biorepository, processed to plasma, blinded with ‘dummy’ subject IDs, and stored at -80°C until being shipped to Guardant Health for testing. Participant results were not returned to participants or providers.

The protocol required participants to undergo bowel preparation and colonoscopy per standard clinical practice within 183 days of the blood draw. Results of the colonoscopy and any further standard of care investigations resulting from the colonoscopy were collected from the sites. Abnormal colonoscopy findings were confirmed and categorized (see Table 5) by central pathologist review.

The study is ongoing and is continuing to follow up participants including but not limited to any additional staging information on the original tumor and participants are being contacted 1 and 2 years after blood sample collection regarding diagnoses of interval malignancies, including both CRC and non-CRC malignancies. The study results are based on the database as of March 2024.

**Figure 10: Location of ECLIPSE Study Sites**



### *5.1.1 Study Objectives and Endpoints*

Co-primary, secondary, and exploratory endpoints in ECLIPSE are shown in Table 4.

**Table 4: ECLIPSE Study Endpoints**

| |
|---|
| **Co-Primary Endpoints** |
| Sensitivity for CRC (performance goal: lower bound of the 2-sided 95% Wilson Confidence Interval > 65%) |
| Specificity for AN (performance goal: lower bound of the 2- sided 95% Wilson Confidence Interval > 85%) |
| **Secondary Endpoint** |
| Sensitivity for AA |
| **Exploratory Endpoints** |
| Positive and negative predictive values |
| Performance by demographic and baseline characteristics |
| Specificity, absent of any neoplastic findings |
| Other malignancies within follow-up window |

AA=advanced adenoma; AN=advanced neoplasia; CRC=colorectal cancer

### *5.1.2 Enrollment Criteria*

Key inclusion criteria included the following:

1. Aged 45 to 84 years at time of consent.
2. Considered by a physician or healthcare provider as being of average risk for CRC.
3. Willing to consent to blood draw pre-bowel preparation administration prior to undergoing colonoscopy.

Key exclusion criteria included the following:

1. Undergoing colonoscopy for investigation of symptoms.
2. Has undergone colonoscopy within preceding 9 years.
3. Positive FIT/fecal occult blood test result within the previous 6 months.
4. Has completed Cologuard or Epi ProColon testing within the previous 3 years.
5. Personal history of CRC.
6. Personal history of any malignancy (participants who have undergone surgical removal of skin squamous cell cancer may be enrolled provided the procedure was completed at least 12 months prior to the date of provision of informed consent for the study).
7. Known diagnosis of inflammatory bowel disease.
8. Currently taking any anti-neoplastic or disease-modifying anti-rheumatic drugs.
9. Family history of CRC, defined as having one or more first-degree relatives (parent, sibling, or child) with CRC at any age.
10. Known hereditary/germline risk of CRC (for example, Lynch syndrome or hereditary nonpolyposis CRC, or familial adenomatous polyposis).

### *5.1.3 Age Group Enrollment Modifications*

Pre-planned modification of enrollment age groups was implemented to enrich for CRC cases and was adjusted throughout the study to meet CRC accrual objectives. The overall intent of the final analysis cohort was to match the final CV cohort to the US age distribution and achieve the target sample size.

For the first 15 months of enrollment, subjects 45 to 84 years of age were recruited. Enrollment was modified to only subjects aged 65 – 84 and was shortly thereafter updated to subjects aged 60 – 84 years of age to increase the prevalence of CRC in the study. Enrollment was opened back up to subjects aged 45+ for the last 4 months of study recruitment.

### *5.1.4 Statistical Analyses*

#### *5.1.4.1 Sample Size Determination*

The study was powered for both co-primary endpoints, and the overall sample size was driven by the number of CRCs needed to power the sensitivity co-primary endpoint.

Target enrollment was 68 evaluable individuals with CRC and 7,000 evaluable individuals negative for AN on colonoscopy. With this sample size target, the study had ≥ 85% power to achieve the co-primary CRC sensitivity endpoint assuming 80.7% true CRC sensitivity, and ≥ 85% power to achieve AN specificity co-primary endpoint assuming 86.3% true AN specificity.

#### *5.1.4.2 Down-Sampling of Non-CRC Participants*

Based on the naturally low prevalence of CRC cases and targeted CRC enrollment sample size, the study was expected to enroll significantly more non-AN participants than the AN specificity co-primary endpoint required. Additionally, the pre-specified enrollment strategy was expected to result in a cohort enriched in older individuals, relative to the intended use population. Based on these 2 factors, the study protocol pre-specified down-sampling the enrolled non-CRC study population prior to testing with Shield to meet the targeted AN specificity sample size and match the age distribution of the 2020 US Census.

#### *5.1.4.3 Interim Futility Analysis*

An Interim Futility Analysis was planned with the intent of stopping the study if the conditional power was insufficient to achieve the co-primary endpoint of AN specificity.

### *5.1.5 Histological Categorization and Analysis*

Local pathology reports were collected for all colonoscopic biopsies. Where multiple lesions were referred for histological examination, the lesion of greatest clinical significance after examination was considered the primary lesion.

Central pathology review of biopsy reports was conducted to consistently categorize lesions as defined in the protocol (see Figure 1). The categories in Table 5 are aligned with other FDA-approved CRC screening tests.

**Table 5: Colonoscopy/Histopathology Diagnosis Category Descriptions**

| Category | Findings |
|---|---|
| 1 | Colorectal cancer, any stage |
| 2 | Advanced adenoma |
| 2a | Carcinoma in situ, any size |
| 2b | High-grade dysplasia, any size |
| 2c | Villous growth % (> 25%), any size |
| 2d | Tubular adenoma, ≥ 10 mm |
| 2e | Serrated lesion, ≥ 10 mm (includes sessile serrated adenoma/polyp) |
| 3 | Non-advanced adenoma, > 3 adenomas, < 10 mm |
| 4 | Non-advanced adenoma, 1 or 2 adenomas, > 5 mm, < 10 mm |
| 5 | Non-advanced adenoma, 1 or 2 adenomas, ≤ 5 mm |
| 6 | Negative, or other findings |
| 7 | Not evaluable |

### *5.1.6 Blinding Procedures*

Study integrity was managed through prospectively defined blinding and data firewalls that separated (a) clinical teams and laboratory teams as well as (b) participant management and plasma sample and testing management. All Guardant Health laboratory and device development staff were blinded to the participants' identities and clinical data, including colonoscopy labels, which were maintained behind the firewall at the independent Contract Research Organization (CRO). The CRO was blinded to all testing results. At a pre-planned time (interim analysis and final analysis), qualitative participant results (Abnormal/Normal/QC failure) and the separate clinical data were combined by an independent analysis team within the CRO for the protocol-specified clinical performance analysis. Only protocol-specified top-level results were returned to Guardant Health.

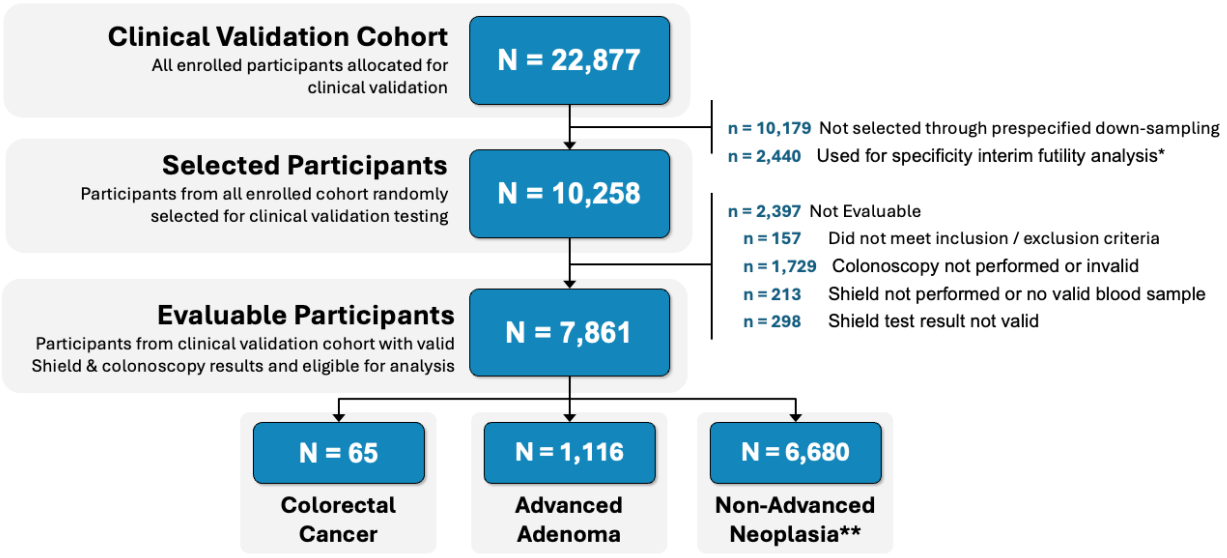
## 5.2 Study Participants

### *5.2.1 Disposition*

Overall, 24,8766 participants were enrolled across 265 clinical sites. A total of 1,999 participants enrolled within a pre-specified time window were allocated to device development and not included in the clinical validation (CV) cohort. (CV). From the 22,877 subjects allocated to the CV cohort, 12,698 were selected for sample testing through either (a) random down-sampling stratified by age to match US Census age distribution and meet the sample size requirements for the AN specificity endpoint or (b) inclusion of all known CRC cases to meet the sample size requirements for the CRC sensitivity endpoint. These 12,698 participants were considered for sample testing. Samples from the remaining 10,179 participants were not tested (Figure 11). Of the selected participants, 2,440 met the prespecified criteria for the Interim Specificity Analysis and were excluded from the primary analysis.

Of the remaining 10,258 CV selected participants, 2,397 were not evaluable. Listing the reasons excluded in a sequential manner were: not meeting study inclusion / exclusion criteria (n=157), lack of a valid colonoscopy result within the protocol defined time window (n=1,729: colonoscopy not performed for n=1,151; not valid per protocol defined procedures for n=537; not performed within 183 days for n=41), lack of an adequate blood sample (n=213), or lack of a valid Shield result (n=298) (Figure 11). Of the 7,861 evaluable participants, 65 had CRC, 1,116 had AA, and 6,680 had non-AN.

**Figure 11: ECLIPSE Participant Disposition**




*4 subjects in the interim futility analysis were determined to not meet I/E criteria
**Non-advanced adenomas, non-neoplastic findings, and negative colonoscopy.

### *5.2.2 Demographics and Baseline Characteristics*

Demographics and baseline characteristics were generally balanced across cohorts and representative of the intended use population. As expected, age distribution was weighted towards older individuals in the CV Cohort; however, stratified down-sampling targeting the US Census age distribution aligned the population of participants selected from the CV Cohort with the expected age in the intended use population. The Evaluable Cohort remained representative across all baseline demographic variables (Table 6).

**Table 6: ECLIPSE Baseline Demographics**

| Characteristic | Clinical Validation (CV) Cohort (N=22,877) | Selected Participants from CV Cohort (N=10,258)* | Evaluable Cohort (N=7,861)* |
|---|---|---|---|
| Age (Years) | | | |
| Mean (SD) | 60.8 (8.26) | 60.6 (9.13) | 60.3 (9.14) |
| Median | 61 | 60 | 60 |
| Min, Max | 22, 90 | 45, 90 | 45, 84 |
| Age Group (Years) | | | |
| 45–49 | 1,890 (8.3) | 776 (7.6) | 640 (8.1) |
| 50–59 | 6,414 (28.0) | 3,877 (37.8) | 3,055 (38.9) |
| 60–69 | 11,185 (48.9) | 3,284 (32.0) | 2,440 (31.0) |
| 70–79 | 3,236 (14.1) | 2,226 (21.7) | 1,670 (21.2) |
| 80+ | 144 (0.6) | 95 (0.9) | 56 (0.7) |
| Sex, n (%) | | | |
| Female | 12,295 (53.7) | 5,493 (53.5) | 4,218 (53.7) |
| Male | 10,581 (46.3) | 4,765 (46.5) | 3,643 (46.3) |
| Race, n (%) | | | |
| American Indian or Alaska Native | 53 (0.2) | 19 (0.2) | 14 (0.2) |
| Asian | 1,868 (8.2) | 685 (6.7) | 560 (7.1) |
| Black or African American | 2,929 (12.8) | 1,353 (13.2) | 931 (11.8) |
| Native Hawaiian or Other Pacific Islander | 48 (0.2) | 24 (0.2) | 19 (0.2) |
| White | 17,431 (76.2) | 7,939 (77.4) | 6,167 (78.5) |
| Other | 440 (1.9) | 189 (1.8) | 137 (1.7) |
| Multiple | 66 (0.3) | 32 (0.3) | 23 (0.3) |
| Ethnicity, n (%) | | | |
| Hispanic | 3,306 (14.5) | 1,561 (15.2) | 1,044 (13.3) |
| Not Hispanic or Latino | 19,460 (85.1) | 8,643 (84.3) | 6,779 (86.2) |
| BMI ($kg/m^2$) at Baseline | | | |
| < 30 | 13,395 (58.6) | 5,873 (57.3) | 4,610 (58.6) |
| ≥ 30 & < 35 | 5,304 (23.2) | 2,460 (24.0) | 1,873 (23.8) |
| 35+ | 4,155 (18.2) | 1913 (18.6) | 1,375 (17.5) |
| Tobacco Use, n (%) | | | |
| Never | 15,920 (69.6) | 7,082 (69.0) | 5,522 (70.2) |
| Current | 2,360 (10.3) | 1,079 (10.5) | 737 (9.4) |
| Former | 4,578 (20.0) | 2,086 (20.3) | 1,601 (20.4) |
| Alcohol Use, n (%) | | | |
| Never | 10,589 (46.3) | 4,673 (45.6) | 3,449 (43.9) |
| Current | 11,033 (48.2) | 4,998 (48.7) | 4,004 (50.9) |
| Former | 1,233 (5.4) | 574 (5.6) | 406 (5.2) |
| Illicit Drug Use, n (%) | | | |
| Never | 21,677 (94.8) | 9,721 (94.8) | 7,481 (95.2) |
| Current | 479 (2.1) | 202 (2.0) | 148 (1.9) |
| Former | 698 (3.1) | 321 (3.1) | 229 (2.9) |

*down-sampling applied to these cohorts
BMI= body mass index; CV=clinical validation; SD=standard deviation.

## 5.3 Interim Futility Analysis

The results of the Interim Futility Analysis passed the futility criteria, and the study continued. The top line results of the specificity analysis informed the decision to update

the threshold for the configuration of the device that contained protein, and therefore participants contributing to the interim analysis AN specificity endpoint (N=2,440) were excluded from final analysis of clinical study endpoints.

## *5.4* Effectiveness Results

### *5.4.1 Co-Primary Endpoints*

ECLIPSE met the performance goals for each of the co-primary endpoints.

Shield detected 54 of 65 colonoscopy identified CRCs for a CRC sensitivity of 83.1% (95% CI: 72.2%–90.3%) (Table 7). This result met the pre-specified performance goal of the lower bound of the 2-sided 95% Wilson's CI exceeding 65%.

Shield AN specificity was 89.6% (95% CI: 88.8%–90.3%). This result met the pre-specified performance goal of the lower bound of the 2-sided 95% Wilson's CI exceeding 85%.

**Table 7: ECLIPSE: CRC Sensitivity and AN Specificity Results**

| | Colonoscopy/Histopathology | | | | |
|---|---|---|---|---|---|
| **Shield Result** | **CRC (N=65)** | **AA (N=1,116)** | **Non-AN (N=6,680)** | **Negative for any Neoplastic Findings (N=4,514)** | **Total (N=7,861)** |
| Abnormal Signal Detected | 54 | 147 | 698 | 457 | 899 |
| Normal Signal Detected | 11 | 969 | 5,982 | 4,057 | 6,962 |
| **Total** | **65** | **1,116** | **6,680** | **4,514** | **7,861** |

| | |
|---|---|
| CRC Sensitivity=% (n/N) (2-sided 95% Wilson CI) | 83.1 (54/65) (72.2%–90.3%) |
| AN Specificity=% (n/N) (2-sided 95% Wilson CI) | 89.6 (5,982/6,680) (88.8%–90.3%) |
| AA Sensitivity=% (n/N) (2-sided 95% Wilson CI) | 13.2 (147/1,116) (11.3%–15.3%) |
| Negative for any Neoplastic Findings Specificity=% (n/N) (2-sided 95% Wilson CI) | 89.9 (4,057/4,514) (89.0%–90.7%) |

AA=advanced adenoma; AN=advanced neoplasia; CI=confidence interval; CRC=colorectal cancer.

### *5.4.2 Secondary Endpoints: Sensitivity for Detection of Advanced Adenoma*

As shown in Table 7, Shield identified 147 of 1,116 individuals with AA for a sensitivity for AA of 13.2% (95% CI: 11.3%–15.3%).

### *5.4.3 Exploratory Analyses*

#### *5.4.3.1 Positive and Negative Predictive Value*

Among all participants who enrolled in the Clinical Validation Cohort of ECLIPSE, met inclusion / exclusion criteria, and had valid histopathological results, the CRC prevalence was 0.41%, and the AN prevalence was 11.2%. At this prevalence, the positive predictive value for CRC was 3.03% (95% CI: 2.7%–3.4%). The CRC positive likelihood ratio is 7.5. The positive predictive value for AN was 17.0% (95% CI: 15.0%–

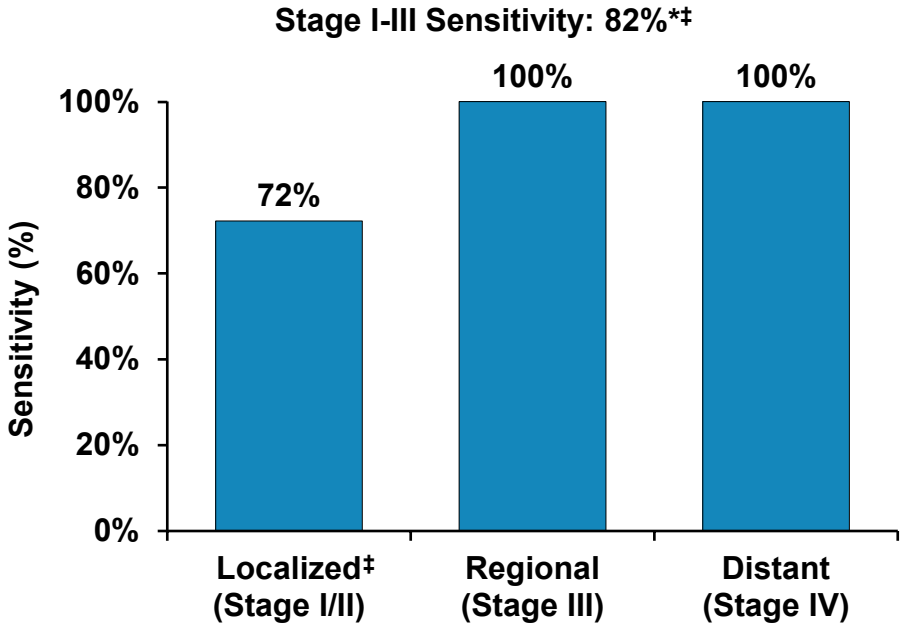
19.1%). The negative predictive value for CRC was 99.9% (95% CI: 99.9%–100.0%).
1.1

*5.4.3.2 Performance by Cancer Stage and Procedural and Lesion Covariates*

Sensitivity for clinical Stage I colorectal cancer was 54.5% (95% CI: 34.7%–73.1%; 12 of 22 cancers), Stage II was 100.0% (95% CI: 78.5%–100.0%; 14 of 14 cancers), Stage III was 100.0% (95% CI: 82.4%–100.0%; 18 of 18 cancers), and Stage IV was 100.0% (95% CI: 70.1%–100.0%; 9 of 9 cancers). Sensitivity for clinical Stage I, II, or III CRC (combined local and regional CRC) was 81.5% (95% CI: 69.2%–89.6%; 44 of 54 cancers) (Table 8; Figure 12). Seven of 65 colorectal cancers did not undergo cancer staging per the AJCC staging; 2 participants were lost to clinical follow-up. The sensitivity in these two individuals was 50.0% (1 of 2 cancers). The remaining 5 cancers were malignant polyps (pT1 submucosal invasive lesions, treated with polypectomy) and staging for N and M was not completed. Malignant polyps are typically managed as clinical Stage I colorectal cancers without undergoing cancer staging and have been included in the Stage I sensitivity results above and in Table 8 (Shaukat et al 2020; Teo et al 2020).

There were no substantial differences in CRC sensitivity by primary tumor location or tumor histologic grade (Table 8). Per-stage sensitivity trended higher with more advanced cancer; however, per-stage sample size was small and did not allow for formal comparison.

**Figure 12: CRC Sensitivity by Stage In ECLIPSE**




*Excludes 2 pathology confirmed, incompletely staged CRCs (sensitivity 1/2; 50%).
‡ Assumes 5 pathology confirmed, incompletely staged CRCs are clinical Stage I CRCs (“malignant polyps”).
Source: Chung et al 2024.

## Table 8: ECLIPSE CRC and AA Sensitivity Based on Key Clinical Features

| | Colorectal Cancer Sensitivity (N=65) | Advanced Adenoma Sensitivity (N=1,116) |
|---|---|---|
| Tumor Location % (n/N) (95% CI) | | |
| Proximal Colon | 88.9 (8/9) (56.5%–98.0%) | 14.5 (92/634) (12.0%–17.5%) |
| Distal Colon | 84.4 (27/32) (68.3%–93.1%) | 10.5 (40/380) (7.8%–14.0%) |
| Rectum | 79.2 (19/24) (59.5%–90.8%) | 14.1 (14/99) (8.6%–22.3%) |
| Missing | - | 33.3 (1/3) (6.2%–79.2%) |
| Most Significant Lesion Size % (n/N) (95% CI) | | |
| < 5 mm | 0.0 (0/1) (0.0%–79.3%) | 0.0 (0/4) (0%–49.0%) |
| 5–9 mm | 0.0 (0/5) (0.0%–43.4%) | 18.8 (9/48) (10.2%–31.9%) |
| 10–19 mm | 87.5 (7/8) (52.9%–97.8%) | 11.9 (102/859) (9.9%–14.2%) |
| 20–29 mm | 83.3 (10/12) (55.2%–95.3%) | 13.6 (18/132) (8.8%–20.5%) |
| ≥ 30+ mm | 94.7 (36/38) (82.7%–98.5%) | 23.6 (17/72) (15.3%–34.6%) |
| Missing | 100.0 (1/1) (20.7%–100.0%) | 100 .0(1/1) (20.7%–100.0%) |
| CRC Tumor Grade % (n/N) (95% CI) | | |
| Grade 1 | 80.0 (4/5) (37.6%–96.4%) | |
| Grade 2 | 80.4 (37/46) (66.8%–89.4%) | |
| Grade 3 | 100.0 (6/6) (61.0%–100.0%) | |
| Missing | 87.5 (7/8) (52.9%–97.8%) | |
| CRC Stage | | |
| I* | 54.5 (12/22) (34.7%–73.1%) | |
| II | 100.0 (14/14) (78.5%–100.0%) | |
| III | 100.0 (18/18) (82.4%–100.0%) | |
| IV | 100.0 (9/9) (70.1%–100.0%) | |
| Stage Unknown | 50.0 (1/2) (9.5%–90.5%) | |

*Assumes 5 incompletely staged by AJCC malignant polyps are Stage I disease
AA=advanced adenoma; CI=confidence interval; CRC=colorectal cancer.

#### *5.4.3.3 Performance by Histology Diagnosis Subcategories*

AA sensitivity trended higher in lesions of greatest malignant potential based pathology features (high-grade dysplasia, 22.6%, and AA with villous component, 17.9%) (Table 9).

**Table 9: ECLIPSE Advanced Adenoma Sensitivity by Histology Diagnosis Subcategories**

| | Advanced Adenoma Sensitivity (N=1,116) % (n/N) (95% CI) |
|---|---|
| Advanced Adenoma Histopathology Diagnosis Subcategories % (n/N) (95% CI)* | |
| Advanced Adenoma, Carcinoma in situ, any size | 0.0 (0/1) (0.0%–79.3%) |
| Advanced Adenoma, with High-grade dysplasia (HGD), any size | 22.6 (7/31) (11.4%–39.8%) |
| Advanced Adenoma with villous component (≥ 25%), any size | 17.9 (37/207) (13.3%–23.7%) |
| Tubular Adenoma ≥ 10 mm in size | 12.0 (82/685) (9.7%–14.6%) |
| Serrated lesion ≥ 10 mm in size | 11.0 (21/191) (7.3%–16.2%) |

*Histopathology on one Advanced Adenoma unknown
CI=confidence interval.

### *5.4.4 Performance by Demographics and Baseline Characteristics*

There was no apparent unexpected variation in performance among subgroups (Table 10). Sensitivity performance was consistent across subgroups based on baseline demographics. Specificity was inversely correlated with age, a trend also observed with other non-invasive CRC screening tests.

## Table 10: ECLIPSE Sensitivity and Specificity by Key Demographic Features

| | CRC Sensitivity (N=65) % (n/N) (95% CI) | AA Sensitivity (N=1,116) % (n/N) (95% CI) | AN Specificity (N=6,680) % (n/N) (95% CI) | Negative Colonoscopy Specificity (N=4,514) % (n/N) (95% CI) |
|---|---|---|---|---|
| Gender | | | | |
| Female | 86.7 (26/30) (70.3%–94.7%) | 13.3 (68/511) (10.6%–16.5%) | 90.1 (3,314/3,677) (89.1%–91.1%) | 90.6 (2,413/2,664) (89.4%–91.6%) |
| Male | 80.0 (28/35) (64.1%–90.0%) | 13.1 (79/605) (10.6%–16.0%) | 88.8 (2,668/3,003) (87.7%–89.9%) | 88.9 (1,644/1,850) (87.4%–90.2%) |
| Age (in years) | | | | |
| 45–59 | 76.5 (13/17) (52.7%–90.4%) | 7.9 (35/441) (5.8%–10.8%) | 93.4 (3,024/3,237) (92.5%–94.2%) | 93.4 (2,161/2,314) (92.3%–94.3%) |
| 60–69 | 88.2 (30/34) (73.4%–95.3%) | 15.1 (63/417) (12.0%–18.9%) | 89.7 (1,785/1,989) (88.3%–91.0%) | 89.6 (1,159/1,293) (87.9%–91.2%) |
| 70+ | 78.6 (11/14) (52.4%–92.4%) | 19.0 (49/258) (14.7%–24.2%) | 80.7 (1,173/1,454) (78.6%–82.6%) | 81.3 (737/907) (78.6%–83.7%) |
| Race | | | | |
| American Indian or Alaska Native | (0/0) | 0.0 (0/2) (0.0%–65.8%) | 83.3 (10/12) (55.2%–95.3%) | 83.3 (5/6) (43.7%–97.0%) |
| Asian | 75.0 (3/4) (30.1%–95.4%) | 17.9 (10/56) (10.0%–29.8%) | 84.4 (422/500) (81.0%–87.3%) | 86.1 (327/380) (82.2%–89.2%) |
| Black or African American | 90.0 (9/10) (59.6%–98.2%) | 13.2 (16/121) (8.3%–20.4%) | 92.1 (737/800) (90.1%–93.8%) | 92.6 (538/581) (90.2%–94.5%) |
| Native Hawaiian or Other Pacific Islander | (0/0) | 0.0 (0/2) (0.0%–65.8%) | 94.1 (16/17) (73.0%–99.0%) | 100.0 (13/13) (77.2%–100.0%) |
| White | 81.6 (40/49) (68.6%–90.0%) | 13.0 (119/917) (11.0%–15.3%) | 89.8 (4,672/5,201) (89.0%–90.6%) | 90.0 (3,080/3,422) (89.0%–91.0%) |
| Other | 100.0 (1/1) (20.7%–100.0%) | 6.3 (1/16) (1.1%–28.3%) | 84.2 (101/120) (76.6%–89.6%) | 84.9 (79/93) (76.3%–90.8%) |
| Multiple | 100.0 (1/1) (20.7%–100.0%) | 50.0 (1/2) (9.5%–90.6%) | 80.0 (16/20) (58.4%–91.9%) | 71.4 (10/14) (45.4%–88.9%) |
| Missing | (0/0) | (0/0) | 80.0 (8/10) (49.0%–94.3%) | 100.0 (5/5) (56.6%–100.0%) |
| Ethnicity | | | | |
| Hispanic | 90.9 (10/11) (62.3%–98.4%) | 18.9 (24/127) (13.0%–26.6%) | 87.3 (791/906) (84.98%–89.32%) | 87.2 (564/647) (84.4%–89.5%) |
| Not Hispanic or Latino | 81.5 (44/54) (69.2%–89.6%) | 12.5 (123/984) (10.6%–14.7%) | 89.9 (5,162/5,741) (89.11%–90.67%) | 90.3 (3,474/3,846) (89.4%–91.2%) |
| Missing | (0/0) | 0.0 (0/5) (0.0%–43.5%) | 87.9 (29/33) (72.67%–95.18%) | 90.5 (19/21) (71.1%–97.4%) |
| Tobacco Use | | | | |
| Never | 82.9 (34/41) (68.7%–91.5%) | 13.4 (95/711) (11.1%–16.1%) | 89.5 (4,269/4,770) (88.59%–90.34%) | 89.8 (2,978/3,316) (88.7%–90.8%) |
| Former | 93.3 (14/15) (70.2%–98.8%) | 13.8 (34/247) (10.0%–18.6%) | 90.2 (1,208/1,339) (88.51%–91.69%) | 90.7 (764/842) (88.6%–92.5%) |
| Current | 66.7 (6/9) (35.4%–87.9%) | 11.4 (18/158) (7.3%–17.3%) | 88.4 (504/570) (85.53%–90.79%) | 88.5 (314/355) (84.7%–91.4%) |
| Missing | (0/0) | (0/0) | 100 (1/1) (20.7%–100.0%) | 100.0 (1/1) (20.7%–100.0%) |

AA=advanced adenoma; AN=advanced neoplasia; CI=confidence interval; CRC=colorectal cancer.

### *5.4.5 Performance on Interval Malignancies During Follow-up*

Data collection and analyses are ongoing and this update from March 2024 has not been fully reviewed by the FDA. As of 4 March 2024, 92% (7,169/7,796) of the evaluable cohort non-CRC participants had 1-year follow-up data available, including 93% (782/845) of participants with colonoscopy categories 2-6 with an abnormal Shield result (CRC false positives) and 92% (6,387/6,951) of individuals with colonoscopy categories 2-6 with a normal Shield result (CRC true negatives). No participants were diagnosed with CRC post-colonoscopy through Year 1. There was no statistically significant difference in the number of participants diagnosed with a non-CRC malignancy in the Shield CRC false positive or true CRC negative groups (1.3% [10/782] versus 0.9% [57/6,387], adjusted p-value=0.539).

For participants with colonoscopy categories 3-6 with an abnormal Shield result (AN false positives), 92% (640/698) of follow-ups were completed and 92% (5,502/5,982) of participants with colonoscopy categories 3-6 with a normal Shield result (AN true negative) were completed. When comparing the number of participants diagnosed with a non-CRC malignancy in the Shield AN false positive group and the Shield AN true negative group, no statistically significant difference is observed (0.8% [5/640] versus 0.9% [51/5,502], adjusted p-value=0.4584).

## 5.5 Safety Evaluation

### *5.5.1 Summary of Adverse Events*

Of the 43 AEs reported in ECLIPSE, 30 (70%) were minor discomfort related to phlebotomy and 13 (30%) were unrelated to the study interventions. No unanticipated adverse device effects (UADEs) were reported.

### *5.5.2 False Positives or False Negatives*

The most impactful anticipated adverse device effects are from potentially inaccurate results, which are classified as false positives or false negatives. False positives in the ECLIPSE study were defined as a positive Shield result in the absence of AN as defined by colonoscopy and histology, and false negatives were defined as a negative Shield result in the presence of CRC as defined by colonoscopy and histology.

False positive results could lead an individual to undergo colonoscopy and the harms associated with that procedure. However, the added risk is minimal given that colonoscopy in this population is recommended even in the absence of an abnormal Shield result. There is no evidence of elevated risk of non-CRC malignancy with false positive Shield results. False negative results could lead individuals with CRC to forgo diagnostic procedures such as colonoscopy. This false negative risk was 17% overall in ECLIPSE, which is within range of other non-invasive CRC screening tests (range: 7–33%), and 0% in Stage II–IV CRC, limiting the false negatives to Stage I and malignant polyps. The long sojourn time (time to clinical detection in the absence of screening) for CRC and the expected high adherence with Shield, combined with high sensitivity for the detection of Stage II localized disease, creates a potential time window to reduce the false negative impact through cumulative sensitivity for CRC detection over time.

# 6 REAL-WORLD ADHERENCE AND CONSIDERATIONS FOR PUBLIC HEALTH OUTCOMES MODELING

**Summary**

- Factoring real world adherence into various test modalities in combination with test performance indicates one-time CRC detection capability for Shield is comparable to that of other guideline-recommended screening tests where lower adherence significantly hinders detection rates.
    - While the real-world adherence to Shield in the first 10,000 clinical tests ordered was 96% for the LDT implementation of Shield, CRC detection is on par with other guideline-recommended screening tests even when considering adherence to Shield as low as 80%.
    - The integration of CRC detection and adherence makes Shield an innovative CRC screening option alongside other long-established screening modalities.
- Screening strategies require repeat testing over time.
    - Health outcomes models are used to estimate population level impact of screening beyond one-time CRC detection.
    - While colonoscopy diversion following the introduction of Shield is not expected at significant levels, public health outcomes remain favorable for CRC deaths averted over current state even if some diversion from colonoscopy were to occur.
- These data suggest that incorporating a simple, convenient blood-based test such as Shield as a screening option can improve screening adherence and lead to more favorable health outcomes.

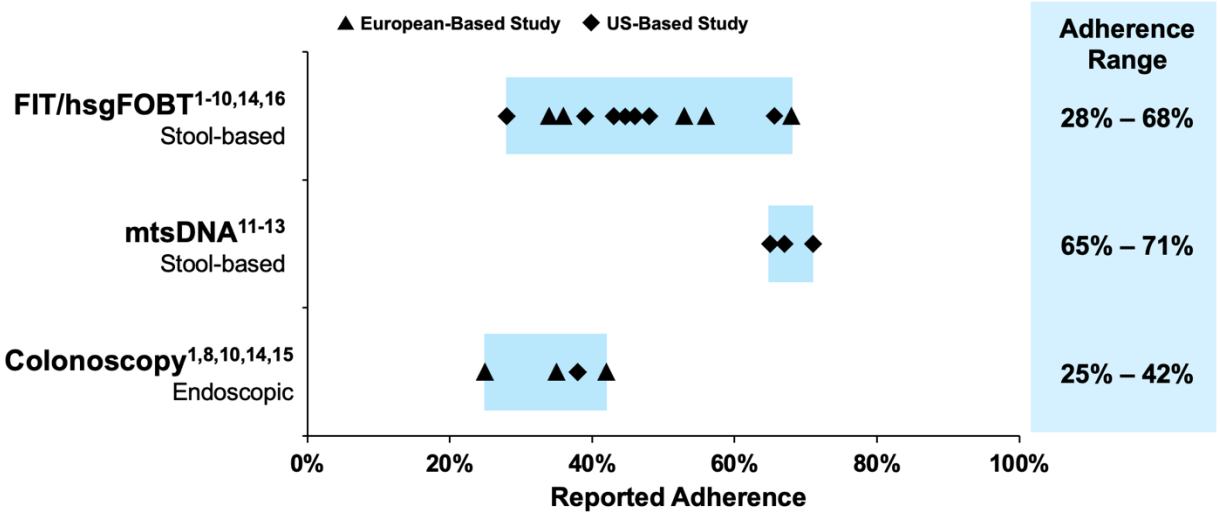
To gain an understanding of the opportunities and challenges of the blood-based CRC screening test, Guardant commercially launched an LDT version of Shield specifically for individuals who are not up to date with their CRC screening. Guardant performed a retrospective review of the first 10,000 clinical orders received for screening age-eligible individuals aimed to define the adherence rate with the test. The adherence for the LDT implementation of Shield was assessed using the number of blood samples received compared with the number of clinical test orders, similar to how adherence is defined and evaluated for stool-based tests in the literature; in addition, a cross-sectional survey was sent to ordering providers and staff to collect data on ordering behaviors (Raymond et al 2023).

Of the 10,000 clinical tests ordered, 9,584 blood samples were received by Guardant Health, which translates to an adherence of 96%. In a survey of ordering practices (N=1,524), 89% of providers shared that they typically ordered Shield for individuals

who were never previously screened or were not up to date with screening, indicating that Shield has the potential for high adherence, even in individuals who are not compliant with currently available CRC screening modalities (Raymond et al 2023). This is the largest dataset reported to date of real-world implementation of blood-based testing, but it has the potential to be skewed based on early adopter bias of new technologies.

In contrast, currently available CRC screening tests have reported lower adherence. An analysis of reported test adherence from high-quality studies published since 2010 is summarized in Figure 13. Of note, due to lack of more recent data, one study of hsgFOBT from 2008 is included. These studies show that stool-based tests have adherence values that range from 28% to 71%, whereas adherence to direct-visualization procedures like colonoscopy and flexible sigmoidoscopy are below 50%.

**Figure 13: Published Adherence for Each Screening Modality**




FIT=fecal immunochemical test; mtsDNA=multi-target stool DNA; US=United States.
Sources: 1. Quintero et al 2012; 2. Jensen et al 2016; 3. Oluloro et al 2016; 4. Binefa et al 2016; 5. Idigoras et al 2017; 6. Bretagne et al 2019; 7. Akram et al 2017; 8. Singal et al 2017; 9. Nielson et al 2019; 10. Forsberg et al 2022; 11. Conroy et al 2018; 12. Weiser et al 2020; 13. Miller-Wilson et al 2021; 14. Inadomi et al 2012; 15. Bretthauer et al 2022. 16. Fenton et al 2010.

When assessing the benefit of a CRC screening option, adherence matters as much as the test's sensitivity. For example, if a test has 100% sensitivity and only 50% of participants complete the prescribed test, i.e. 50% adherence, only half of the individuals who have colorectal cancer would be identified. Considering the reported CRC sensitivity rates in Table 3 and factoring in the range of adherence in Figure 13, the estimated one-time CRC detection probability, even for a highly sensitive test such as colonoscopy, is meaningfully reduced as shown in Table 11. Even when assessing Shield at an adherence of 80%, which is below the value observed in the first 10,000 patients tested with the LDT implementation, CRC detection remains at or above that of other CRC screening testing modalities (66%). This is true also when considering the impact of adherence on the detection probability of advanced adenomas (Table 13).

Thus, the accuracy of a test and an individual's willingness to undergo it are equally important in assessing potential benefits of a new screening option at a population level.

**Table 11: One-Time CRC Detection Rate for Available Screening Modalities Based on CRC Sensitivity and One-Time Adherence Described in the Literature**

| Screening Modality | CRC Sensitivity | One-Time Adherence | One-Time CRC Detection Probability (CRC Sensitivity x Adherence) |
|---|---|---|---|
| Colonoscopy | 95%[1] | 25–42%[5-9] | 24–40% |
| mtsDNA | 92–94%[2,3] | 65–71%[10-12] | 60–67% |
| FIT | 67–74%[2,3] | 28–68%[5-7, 13-19] | 19–50% |
| hsgFOBT | 50–75%[21,22] | 44–67%[8,23] | 22–50% |
| Shield | 83%[4] | 90–96%[20] | 75–80% |

CRC=colorectal cancer; FIT=fecal immunochemical test; mtsDNA=multi-target stool DNA; hsgFOBT=high-sensitivity guaiac-based fecal occult blood test

Sources: 1. Pickhardt et al 2011; 2. Imperiale et al 2014; 3. Imperiale et al 2024; 4. Chung et al 2024; 5. Quintero et al 2022; 6. Singal et al 2017; 7. Forsberg et al 2022; 8. Inadomi et al 2012; 9. Bretthauer et al 2022; 10. Conroy et al 2018; 11. Weiser et al 2020; 12. Miller-Wilson et al 2021; 13. Jensen et al 2016; 14.Oluluro et al 2016; 15. Binefa et al 2016; 16. Idigoras et al 2017; 17. Bretagne et al 2019; 18. Akram et al 2017; 19. Nielson et al 2019; 20. Raymond et al 2023; 21. Shapiro et al 2017; 22. Ahlquist et al 2008; 23. Fenton et al 2010.

**Table 12: One-Time AA Detection Rate for Available Screening Modalities Based on AA Sensitivity and One-Time Adherence Described in the Literature**

| Screening Modality | AA Sensitivity | One-Time Adherence | One-Time AA Detection Probability (AA Sensitivity x Adherence) |
|---|---|---|---|
| Colonoscopy | 95%[1] | 25–42%[5-9] | 24–40% |
| mtsDNA | 42–43%[2,3] | 65–71%[10-12] | 27–31% |
| FIT | 23–24%[2,3] | 28–68%[5-7, 13-19] | 6–16% |
| hsgFOBT | 6–17%[21,22] | 44–67%[8,23] | 3-11% |
| Shield | 13%[4] | 90–96%[20] | 12% |

AA=Advanced Adenoma; FIT=fecal immunochemical test; mtsDNA=multi-target stool DNA. hsgFOBT=high-sensitivity guaiac-based fecal occult blood test

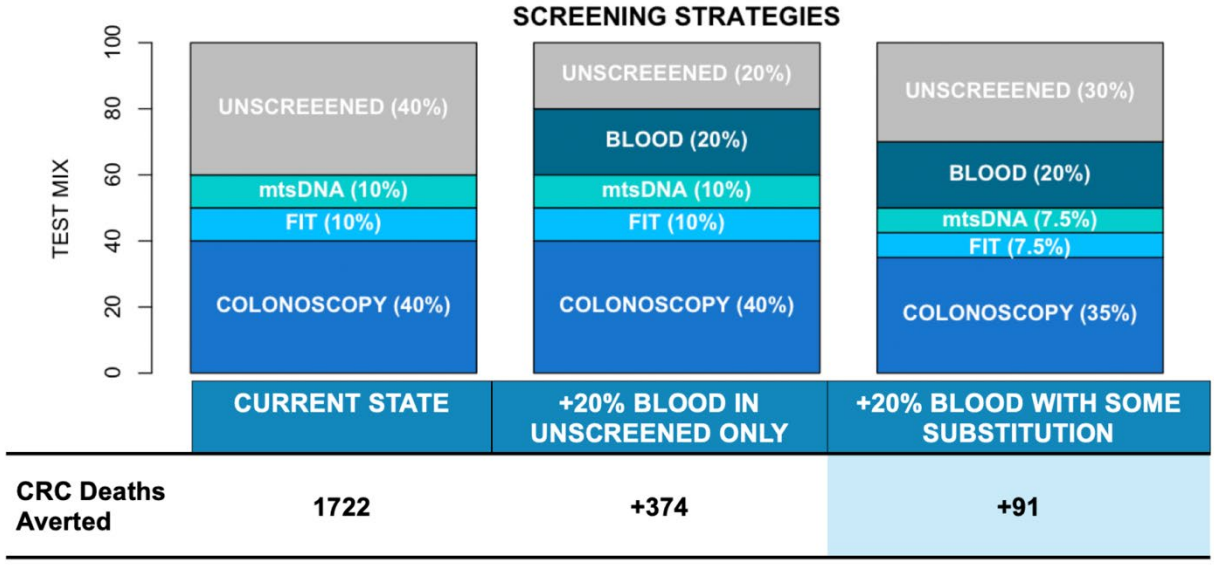
Sources: 1. Pickhardt et al 2011; 2. Imperiale et al 2014; 3. Imperiale et al 2024; 4. Chung et al 2024; 5. Quintero et al 2022; 6. Singal et al 2017; 7. Forsberg et al 2022; 8. Inadomi et al 2012; 9. Bretthauer et al 2022; 10. Conroy et al 2018; 11. Weiser et al 2020; 12. Miller-Wilson et al 2021; 13. Jensen et al 2016; 14.Oluluro et al 2016; 15. Binefa et al 2016; 16. Idigoras et al 2017; 17. Bretagne et al 2019; 18. Akram et al 2017; 19. Nielson et al 2019; 20. Raymond et al 2023; 21. Shapiro et al 2008; 22. Ahlquist et al 2008; 23. Fenton et al 2010.

While the analysis shown in Table 12 is informative for assessment of one-time CRC detection and highlights the value of adherence when evaluating test effectiveness, it does not fully address population-level outcomes based on repeat testing, the test performance in AA and CRC detection, and CRC disease development over a lifetime. Health outcomes models are used to estimate population-level impact of screening strategies beyond one-time CRC detection. Recently published models by CISNET have shown a reduction in CRC mortality through serial blood-based testing when

compared to no screening. The CISNET models also evaluated the impact of a blood-based test with CRC detection similar to that of FIT (74%, lower than that of Shield) against guideline-recommended strategies (van den Puttelaar et al 2024). The evaluation of clinical outcomes found that a blood-based test with screening participation of 80% resulted in CRC mortality reduction similar to stool-based testing with screening participation of 60%. This demonstrates the importance of incorporating adherence in assessing public health impact. The finding is consistent with results from a health outcomes model developed by Guardant Health that used real-world adherence (based on rate of test ordered versus test completed) rather than screening strategy participation as modeled by CISNET (Appendix 9.2).

A question for the integration of a blood-based test to be offered alongside currently available screening tests is how the population-level test mix may be altered. One possibility is that individuals may opt not to change from their preferred screening modality and adoption of Shield occurs only among those who are not up to date with CRC screening. Another possibility is that individuals who may have otherwise elected to complete colonoscopy or non-invasive stool-based testing instead elect Shield. Data from the introduction of mtsDNA as a testing modality indicates that diversion from colonoscopy is unlikely to occur at significant levels (Fisher et al 2021). A modeling analysis performed by Ladabaum et al evaluated the impact of introducing a blood-based test where there is some diversion from existing screening modalities to a blood-based test with CRC detection similar to that of FIT (74%, lower than that of Shield). In the current state scenario that was modeled (as shown in the first column of Figure 14), 40% of individuals were currently unscreened, 40% were screened by colonoscopy, 10% were screened by FIT, and 10% were screened by mtsDNA. In a hypothetical diversion scenario (as shown in the third column of Figure 14), there was 20% uptake of a blood-based test with 10% in those unscreened and 10% in those who would have undergone screening with another modality (split across diversion of 25% of individuals currently screened with stool-based tests and 12.5% diversion of individuals who are currently screened with colonoscopy). Even with this level of diversion, this test mix yields CRC mortality reduction over the current state scenario (Ladabaum et al 2024).

**Figure 14: Evaluation of CRC Deaths Prevented When Blood-based testing (with FIT-Like CRC Sensitivity of 74%, 90% AN Specificity, and 10% AA Sensitivity) is Introduced Alongside Other Screening Modalities**




Adapted from Table 3 of Ladabaum et al 2024.

The analysis of public health outcomes demonstrates that having a blood-based test such as Shield as a screening option, with clinically meaningful performance and high participant adherence over their lifetime, will lead to favorable health outcomes that are comparable to existing recommended modalities.

# 7 BENEFIT-RISK CONCLUSIONS

## 7.1 Benefits

The goal of CRC screening is to reduce CRC-related mortality. Empirical data from clinical trials of guaiac FOBT and flexible sigmoidoscopy, which have test performance similar to that of Shield, demonstrate a reduction in CRC-related mortality. CRC is known to be a relatively slow growing disease, providing multiple opportunities to intervene during the disease course to achieve this goal. However, adherence to currently available screening modalities is inadequate, and despite availability of colonoscopy, introduction of stool tests with improved performance over a decade ago, and significant public health educational efforts, screening rates remain below goals set by leading public health organizations.

To achieve the goal of CRC mortality reduction through screening, it is important to consider that the clinical benefit of a CRC screening test depends equally on both test performance and adherence, or the likelihood a person will complete the test. In other words, the probability of identifying an existing cancer is equally dependent on the completion of the test and the sensitivity of the test.

For this reason, a screening intervention must not only demonstrate clinical validity, but must also consider an individual's preference for acceptability and accessibility to achieve the participation necessary for population-level benefit (Bretthauer et al 2022; Goding Sauer et al 2019; Kurani et al 2020; Singal et al 2014; Singh and Jemal 2017; World Health Organization 2020). For example, colonoscopy has the advantage of the highest sensitivity of all screening modalities, but its clinical effectiveness is limited by poor real-world adherence (Bretthauer et al 2022). In contrast, higher adherence to accurate blood-based testing combined with device performance within range of existing stool-based options has the potential to improve overall screening rates and impact CRC-related mortality.

In the ECLIPSE study, Shield demonstrated CRC sensitivity of 83% and AN specificity of 90%. These key performance metrics are within range of currently recommended non-invasive stool-based tests (Table 13) and superior to the previously FDA-approved mSept9 blood-based test. Shield's sensitivity for each of the CRC stages is within range of or higher than that of the most commonly utilized colonoscopy alternative, FIT, and Shield has 100% sensitivity for Stage II–IV disease.

In addition, data suggest adherence to Shield to be upwards of 90%, far outpacing the adherence to currently available endoscopic and stool-based non-invasive screening options, which range from 25% to 71%. Evidence from a patient preference study shows that a blood-based test will improve CRC screening adherence if included in screening recommendations and offered alongside other non-invasive CRC screening options (Schneider et al 2023).

The demonstrated performance of Shield for CRC detection combined with the observed high adherence supports Shield's utility as a primary CRC screening option

that individuals will complete, resulting in the potential to improve clinical outcomes. Independently published health outcomes modeling data suggest that a blood-based test with CRC performance similar to that of FIT (74%, lower CRC sensitivity than that of Shield) and observed adherence make the clinical effectiveness comparable to other currently available CRC screening tests.

## 7.2 Risks

### *7.2.1 Risk from an Inaccurate Shield Result*

The risk from an inaccurate Shield result is within range with guideline recommended non-invasive screening tests. Table 13 and Figure 15 display currently available stool-based non-invasive CRC screening tests as well as the mSept9 blood test. Shield's CRC sensitivity (both overall and for early-stage cancer) and AN specificity are within range with those of FIT and mtsDNA, indicating the indirect risks related to the false negative and false positive results are on par with current guideline-recommended non-invasive primary screening methods.

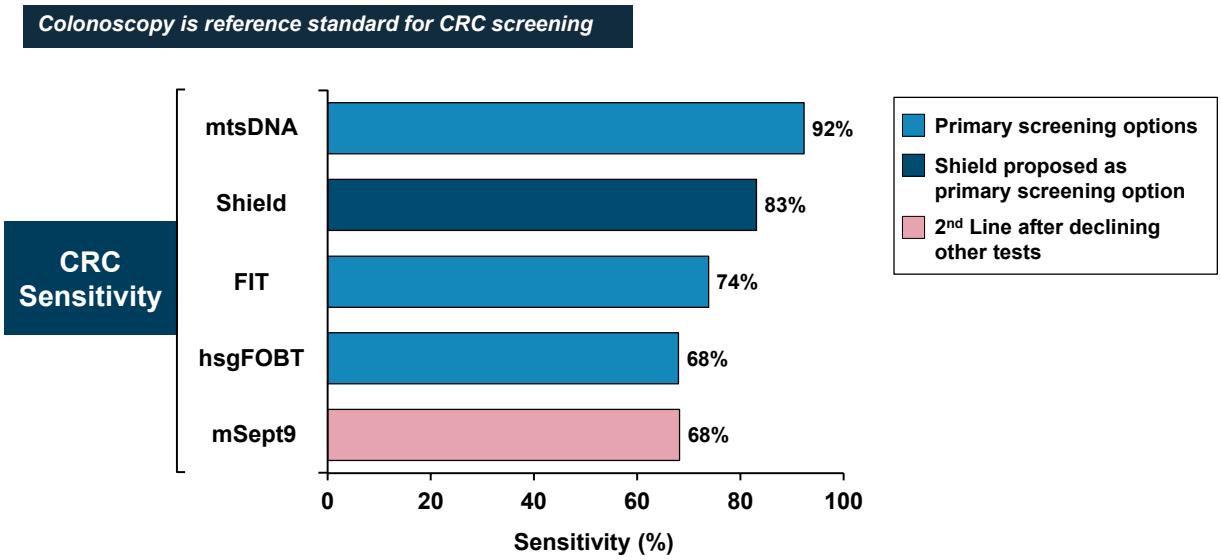
**Table 13: Performance of Shield and Existing Non-Invasive CRC Screening Tests**

| | | Primary Non-Invasive CRC Screening Tests# | | | | 2nd Line (After Declining Other Tests) |
|---|---|---|---|---|---|---|
| | | mtsDNA % (n/N) | Next-generation mtsDNA† % (n/N) | Shield % (n/N) | FIT % (n/N) | mSept9‡ % (n/N) |
| Colon Cancer Detection | Overall CRC Sensitivity | 92.3% (60/65)[1] | 93.9% (92/98) [2] | 83.1% (54/65)[4] | 73.8% (48/65)[1]<br>67.3% (66/98)[2] | 68.2% (30/44)[3] |
| | Localized CRC (Stage I/II) | 94.0% (47/50)[1] | 89.6% (43/48)[2] | 72.2% (26/36)[4+] | 70.0% (35/50)[1]<br>60.4% (29/48)[2] | 58.6% (17/29)[3] |
| | Regional CRC (Stage III) | 90.0% (9/10)[1] | 97.1% (33/34)[2] | 100.0% (18/18)[4] | 90.0% (9/10)[1]<br>70.6% (24/34)[2] | 80.0% (8/10)[3] |
| | Metastatic CRC (Stage IV) | 75.0% (3/4)[1] | 100.0% (12/12)[2] | 100.0% (9/9)[4] | 75.0% (3/4)[1]<br>83.3% (10/12)[2] | 100.0% (5/5)[3] |
| | Early-Stage CRC (Stage I-III) | 93.3% (56/60)[1] | 92.7% (76/82)[2] | 81.5% (44/54)[4+] | 73.3% (44/60)[1]<br>64.6% (53/82)[2] | 64.1% (25/39)[3] |
| Specificity | AN Specificity | 86.6% (7,936/9,167)[1] | 90.6% (16,245/17,934)[2] | 89.6% (5,982/6,680)[4] | 94.9% (472/9,167)[1]<br>94.8% (16,997/17,934)[2] | 79.1% (695/879)[3] |

#hsgFOBT & computed tomography colonography are also non-invasive guideline recommended screening strategies, which are not included in the table above due to their relatively low usage.[5]
†Currently not yet commercialized.[6]
‡Discontinued marketing.[7]

+Presumes 5 individuals with pathology confirmed, incompletely staged malignant polyps are clinical Stage I cancer.
AN=advanced neoplasia; CRC=colorectal cancer; FIT=fecal immunochemical test; mSept9=methylated Septin 9; mtsDNA=multi-target stool DNA.
Sources: 1. Imperiale et al 2014; 2. Imperiale et al 2024; 3. Potter et al 2014; 4. Chung et al 2024; 5. Fisher et al 2021; 6. Exact Sciences 2024; 7. Epigenomics 2023.

**Figure 15: CRC Sensitivity of Shield and Existing Non-Invasive CRC Screening Tests**




CRC=colorectal cancer; FIT=fecal immunochemical test; mSept9=methylated Septin 9; mtsDNA=multi-target stool DNA.
Note: mSept9 offered after refusal of other options (colonoscopy, FIT, mtsDNA); termed "2nd line"

While a false positive could lead to an unnecessary colonoscopy, colonoscopy is a recommended screening intervention in this population, thus individuals with a false positive Shield are not incurring additional risk. Evaluation of clinical follow-up of participants enrolled in ECLIPSE showed that the risk of a non-colorectal cancer in an individual with a false positive Shield result to be no different than those with a true negative result.

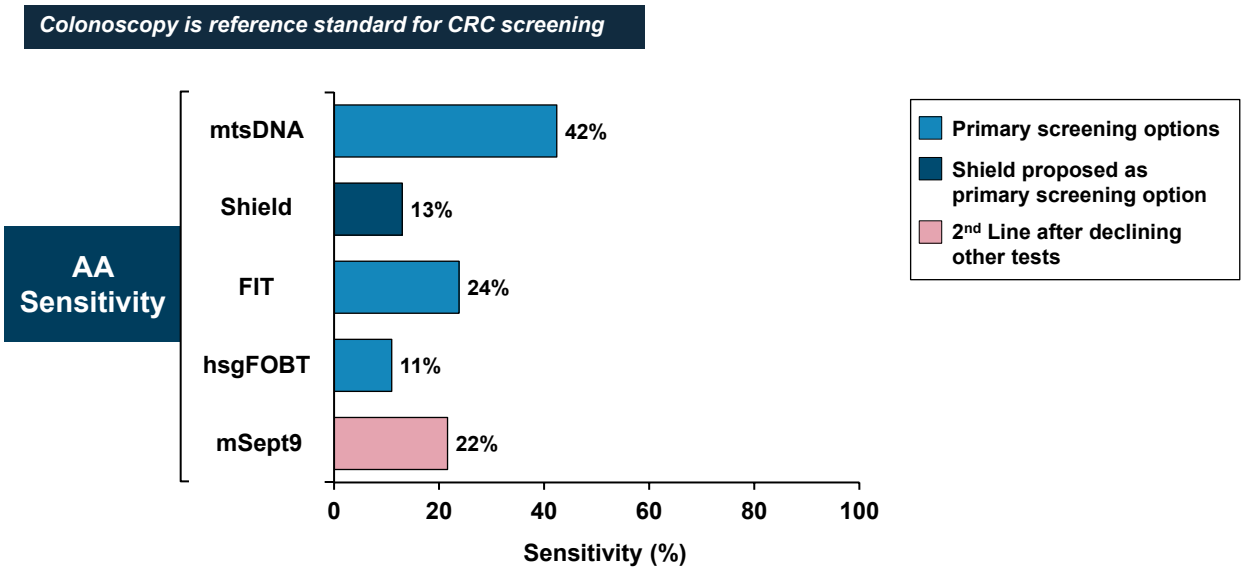
The risk of a false negative result is quantified in the ECLIPSE study through both the false negative rate and the negative predictive value, which indicate that fewer than 1 in 1,000 tested individuals would have undetected CRC. However, the observed CRC sensitivity of 83% means that 17% of individuals with CRC on colonoscopy are expected to be negative on a one-time Shield test. Given patient preference for non-invasive methods, this decrement in sensitivity might increase an individual's exposure to the risk of false negatives if Shield were chosen in lieu of colonoscopy. However, the increased risk attributable to Shield in this scenario is not greater than observed in existing testing strategies, as multiple non-invasive CRC screening tests with performance characteristics similar to Shield (Table 13) are in routine use today as primary screening tests. Specifically, the false negative rate observed in ECLIPSE, 17%, is within range of the false negative rates for existing non-invasive CRC screening tests, 7-33%. The false negative rate was 0% in Stage II–IV CRC, limiting the false

negatives to Stage I and malignant polyps. The extended dwell time from adenoma to CRC, and the frequency of repeat testing with Shield allows for multiple opportunities to intervene along the disease course with minimal accrued harm. The expected improvement in adherence to the first screening test and subsequent repeat testing with Shield in conjunction with device performance within range of existing primary screening methods minimizes the risk of significant harm resulting from a false negative result.

### *7.2.2 Risk Related to Limited Advanced Adenoma Detection*

Shield has a limited ability to detect advanced adenomas, with a sensitivity of 13% as compared to colonoscopy. Stool-based testing also has limited detection (24–42%, Table 3) for these lesions (Figure 16). Detection of advanced adenomas can prevent the development of CRC and impact CRC incidence and reduce mortality. CRC mortality reduction can also be achieved by detection of CRCs at an early asymptomatic stage while the disease is treatable. The lower test adherence with stool-based testing further reduces the one-time AA detection when individuals are prescribed those tests, to the range of 6-16% for FIT and 27-30% for mtsDNA using adherence assumptions (). Shield's limited advanced adenoma detection could result in harm if the screening test is used only once in a lifetime. Given Shield's expected adherence rate and the extended dwell time of adenomas to CRC, the risk of limited advanced adenoma detection and expected accrued harm would be minimal. Shield is expected to improve overall population CRC screening rates, which potentially may outweigh the harm from individuals pursuing Shield over other CRC screening options.

**Figure 16: Advanced Adenoma Sensitivity for Non-Invasive CRC Screening Tests**




mSept9: offered after refusal of other options (Colonoscopy, FIT, mtsDNA); termed "2nd line"

Shield can impact CRC prevention and incidence if individuals who otherwise would opt to complete colonoscopy instead select Shield as their CRC screening option. This is

highly unlikely to occur as other non-invasive CRC screening tests have been available for more than a decade, and displacement of colonoscopies has not been observed. Nonetheless, outcomes modeling suggests a blood-based test with CRC performance similar to that of FIT (74%) will lead to overall outcomes by improving population CRC screening rates, even if 50% of individuals who elect to complete a blood-based test would have otherwise chosen another screening modality (Ladabaum et al 2024). This population-level benefit outweighs the harm associated with the burden associated with using blood tests in second-line indication.

### *7.2.3 Risks Related to Diversion from Colonoscopy*

Guideline-recommended non-invasive screening tests are used broadly today, with the selection of which test and/or colonoscopy being driven by patient and provider preference after discussing the benefits and limitations of each test and the likelihood an individual will complete each test. Individuals undergoing colonoscopy today, even with widespread availability of existing non-invasive CRC screening tests, are unlikely to change behavior. This observation is confirmed by a study of population-level screening patterns following the introduction of mtsDNA in which no significant impact on the rate of CRC screening with colonoscopy was observed (Fisher et al 2021). There is no expectation that this effect will be different with the introduction of Shield as another primary non-invasive screening test and colonoscopy will remain the reference screening test for adherent patients who have access to it. Findings of an independently published modeling analysis showed that incorporating a blood-based test with CRC performance similar to that of FIT (74%) (lower CRC sensitivity performance than that of Shield) demonstrated CRC mortality reduction even with some level of displacement of existing tests (Ladabaum et al 2024).

To mitigate the risk of diversion from colonoscopy, Guardant has proposed a risk mitigation strategy that includes physician and provider education to clearly outline the benefits and limitations of Shield, including patient friendly language on device performance, implications of a “false positive” or a “false negative” result, the need for repeat testing at regular intervals in people who have a “Normal Signal Detected”, and the need for diagnostic colonoscopy in those with an “Abnormal Signal Detected”. The goal is to allow physicians to employ the test most appropriate for the individual and the one the individual is most likely to complete. Given the shared non-invasive nature and similar performance characteristics, Shield is expected to follow the established precedent of other primary non-invasive CRC screening tests to allow physicians to direct individuals to the most appropriate test. Guardant is committed to working with the FDA and clinical guideline committees to generate evidence-based recommendations on incorporation of Shield into medical practice.

## 7.3 Benefit-Risk Analysis

CRC screening saves lives, yet 42% of eligible Americans are not up to date with screening programs. Currently available screening modalities are burdensome, inconvenient, and have low adherence, resulting in 50 million eligible adults who are not

up to date with screening and therefore are at increased risk for CRC. Shield addresses the unmet need in CRC screening by providing a convenient, blood-based screening test that can be completed during a routine doctor's visit, improving accessibility and adherence to CRC screening, and providing the ability to complete CRC screening at the clinical point of care without increasing risk to individuals.

Findings from the pivotal study, ECLIPSE, demonstrate that Shield is the first blood-based test with performance within range of other primary non-invasive CRC screening tests. Advanced adenoma detection is limited; however, given the multi-year dwell time for adenoma to CRC and Shield's high sensitivity for CRC, this allows for multiple time points for detection before individuals are at high risk for mortality, thus accruing minimal additional harm. The indirect risks of false positive and false negative results are aligned with other primary non-invasive screening options.

### *7.3.1 Benefit-Risk of Limited Advanced Adenoma Detection*

Shield, like other non-invasive CRC screening tests, has limited ability to detect advanced adenomas relative to colonoscopy. Detection of these lesions can prevent the development of CRC, and thus impact CRC incidence. However, when considering the multi-year transition from advanced adenoma to CRC, which allows multiple opportunities for testing, Shield's high CRC sensitivity for detecting early-stage CRCs including 100% sensitivity in Stage II and III, and increased adherence to the Shield blood tests, the risk of accrued harm is mitigated. Additionally, at a public health level, including Shield as a primary CRC screening option, alongside existing options, yields benefits of improving overall population CRC screening rates.

### *7.3.2 Benefit-Risk Related to Diversion from Colonoscopy*

Like other non-invasive CRC screening tests, Shield has the potential to divert individuals from colonoscopy to lower-sensitivity tests. The incremental risk from approval of Shield is minimal as Shield has performance within range of currently available stool-based CRC screening tests. A label approving Shield to only be offered after declining other screening options is not appropriate as it would introduce an artificial non-performance-based distinction between existing non-invasive screening tests that would result in loss of patient access to the benefits of blood-based testing. This barrier would significantly hinder clinical adoption of new CRC screening tests and limit access for individuals who would benefit. Data consistently show that incorporating choice between multiple CRC screening options improves the rate of screening completion. Empowering physicians and patients with multiple guideline-recommended CRC screening tests will increase the likelihood the individual will opt for the test they are likely to complete instead of agreeing to an option that they later will not complete. The benefits of improved CRC screening completion rates by including Shield as a primary CRC screening option alongside currently available stool-based tests far outweigh any potential harms from individuals selecting Shield over other options.

### *7.3.3 Overall Benefit-Risk Profile for Shield*

Shield has CRC sensitivity and specificity within the range of other guideline recommended non-invasive primary CRC screening tests, including FIT, hsgFOBT, and mtsDNA and above that of current FDA approved blood-based test (Table 13, Figure 16). As such, it is appropriate for Shield to be approved for primary (first-line) use for average risk individuals and the evidence demonstrates the clinical value of Shield in the proposed intended use. Incorporating Shield alongside the other guideline-recommended CRC screening options empowers physicians and their patients to complete CRC screening, bringing the benefit of selecting the most appropriate test for individuals and helping to achieve the 80% target set forth by the leading public health organizations, and reduce CRC mortality. Given the totality of evidence, this provides an additional and much needed opportunity to impact the second leading cause of cancer-related mortality – colorectal cancer. The performance of Shield as shown here demonstrates that the benefits of Shield as a primary CRC screening option outweigh the risks. Shield will fill an important gap in CRC screening options and has a favorable benefit-risk profile.

## 8 REFERENCES

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# 9 APPENDICES

## 9.1 Shield Performance Estimate Robustness

### *9.1.1 Assessment of AN Specificity in the Interim Analysis Specificity Cohort*

We assessed the robustness of the AN specificity co-primary endpoint estimate with respect to the exclusion of 2,440 participants ('Excluded Cohort') contributing to the Interim Futility Analysis AN Specificity assessment from the Evaluable Cohort used to derive primary performance estimates. This was done by evaluating AN specificity in the Excluded Cohort in comparison to that observed in the Evaluable Cohort used to derive the co-primary AN specificity endpoint. Given the observed dependence of specificity on age, this analysis was stratified by age and final estimates were age-adjusted to bring the excluded cohort into alignment with that used for the primary analysis.

While 2,440 participants were excluded at the time of interim analysis, 4 of those participants were found to not meet study inclusion criteria based on the clinical database freeze at the time of primary analysis. These 4 participants were excluded from the specificity analysis presented in Table 14.

AN specificity in the Excluded Cohort was 89.1% (95% CI: 87.8%–90.3%), with the lower bound of the 95% CI of 87.8% exceeding the co-primary endpoint performance goal of 85%. The age-adjusted estimate of AN specificity was 90.3%.

This comparison of AN specificity demonstrates similar performance in the Evaluable Cohort and in the Excluded Cohort, confirming that exclusion of these participants did not meaningfully alter ECLIPSE performance estimates.

**Table 14: Specificity Estimates Stratified by Age Within Evaluable Cohort and the Interim Analysis Specificity Cohort for Participants Meeting Study Inclusion/Exclusion Criteria**

| Age Group | Evaluable Cohort N=6,680 | Interim Analysis Specificity Cohort, Meeting Study Inclusion/Exclusion Criteria N=2,436 |
|---|---|---|
| **All** | **89.6%**<br>(88.8%–90.3%)<br>5,982/6,680 | **89.1%**<br>(87.8%–90.3%)<br>2,171/2,436 |
| **45–49** | **95.5%**<br>(93.5%–96.9%)<br>554/580 | **98.0%**<br>(89.5%–99.6%)<br>49/50 |
| **50–59** | **93.0%**<br>(91.9%–93.9%)<br>2,470/2,657 | **94.0%**<br>(91.6%–95.7%)<br>497/529 |
| **60–69** | **89.7%**<br>(88.3%–91.0%)<br>1,785/1,989 | **89.3%**<br>(87.6%–90.8%)<br>1,271/1,423 |
| **70–79** | **80.9%**<br>(78.7%–82.8%)<br>1,136/1,405 | **82.0%**<br>(78.0%–85.4%)<br>333/406 |
| **80+** | **75.5%**<br>(61.9%–85.4%)<br>37/49 | **75.0%**<br>(56.6%–87.3%)<br>21/28 |
| **Age Adjusted** | **N/A** | **90.3%*** |

Note: The values in each cell include the point estimate (first row), the 95% 2-sided Wilson CI (second row), and the number of correct detection results/total (third row).
*The values for Age Adjusted AN Specificity for n = 2.436 participants were calculated by adjusting AN specificity to the age distribution observed in the Evaluable Cohort.
AN=advanced neoplasia; CI=confidence interval; N/A=not applicable.

## 9.2 Public Health Outcomes Model To Assess the Multiple Factors That Impact the Benefits of Screening

The results of the public health outcomes models have not been fully reviewed by the FDA. Given the marked difference in adherence to the various CRC screening tests and the subsequent impact on real-world CRC detection as demonstrated in Table 12, the availability of a blood-based offered alongside other screening modalities has the potential to increase CRC screening adherence and improve public health outcomes. While the analysis shown in Table 12 is informative for assessment of one-time CRC detection and highlights the value of adherence when evaluating test effectiveness, it does not fully address population-level outcomes based on repeat testing, the test performance in detecting AA and CRC, and CRC disease progression over a lifetime. To assess the public health impact of Shield as a blood-based screening option and account for differences in adherence to the test modality, Guardant designed a discrete-event public health outcomes model (Colorectal cANcer SCReening Economics and adherence, or CAN-SCREEN), which can be used to compare the effectiveness of lifetime screening with Shield and other screening options (Forbes et al 2023). The CAN-SCREEN model includes the natural history of CRC progression based on rates of

adenoma initiation, adenoma growth, and the transition to preclinical CRC and symptomatic CRC. The test performance characteristics of colonoscopy, FIT, and mtsDNA used in the CAN-SCREEN model are provided in Table 15.

**Table 15: CAN-SCREEN Model Inputs**

| | CRC Sensitivity | AN Specificity | AA Sensitivity | Screening Interval | Adherence |
|---|---|---|---|---|---|
| **Colonoscopy** | 95% | 86% | 85% | 10 years | 38%[1] |
| **FIT** | 74% | 96% | 24% | 1 year | 43%[2] |
| **mtsDNA** | 92% | 90% | 42% | 3 years | 65%[3] |
| **Shield** | 83% | 90% | 13% | 3 years | 90%[4] |

AA=advanced adenoma; AN=advanced neoplasia; CRC=colorectal cancer; FIT=fecal immunochemical test; mtsDNA=multi-target stool DNA.
Sources: 1. Singal et al 2017; 2. Akram et al 2017; 3. Conroy et al 2018; 4. Raymond et al 2023.

After model calibration and validation against public health benchmarks and modelling results used by the USPSTF were completed, Guardant incorporated adherence into the CAN-SCREEN model based on a real-world longitudinal adherence scenario (D'Andrea et al 2020). As provided in Table 15, the initial point-estimate chosen to model longitudinal adherence was 38% for colonoscopy, 43% for FIT, 65% for mtsDNA, and 90% for Shield. The testing interval was set to annual testing for FIT, 3-year interval testing for mtsDNA, and 10-year for colonoscopy. For Shield, a 3-year interval was modeled. Compliance with diagnostic colonoscopy following a positive stool- or blood-based test result was set to 56.1% for all non-invasive tests, based on a finding reported by Mohl et al (Mohl et al 2023). Each screening strategy was evaluated by 1,000 trials of the CAN-SCREEN model using a cohort of 10,000 individuals, and the average of all replicates was used to calculate outcomes.

The outputs of the CAN-SCREEN model under these assumptions were used to assess health outcomes and resource utilization for each screening modality. The results are presented in Table 16. Compared with all other screening modalities, the CAN-SCREEN model found that colonoscopy averted the most CRC deaths. However, the number of CRC deaths that were averted in the Shield testing scenario was higher than those of stool-based testing (12 vs 7 and 9), demonstrating population health impact and individual level benefits.

**Table 16: Lifetime Outcomes per 1,000 Simulated Individuals Offered Interval Screening With Shield and Other Screening Modalities Based on the CAN-SCREEN Model Using Longitudinal Adherence**

| Screening Modality | Life Years Gained (LYG) (per 1000) | CRC deaths averted: Estimated Lifetime Risk of Events (per 1000) | Number of Lifetime Colonoscopies (per 1000) |
|---|---|---|---|
| Colonoscopy | 255 | 15 | 1996 |
| FIT | 120 | 7 | 107 |
| mtsDNA | 112 | 9 | 450 |
| Shield | 151 | 12 | 655 |

CRC=colorectal cancer; FIT=fecal immunochemical test; mtsDNA=multi-target stool DNA.